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Evaluating the role of stress and parasite load in sarcoptic mange incidence in bare-nosed wombats (*Vombatus ursinus*) in N.S.W., Australia

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Statement of Authenticity

This thesis is composed of my original work, and contains no material previously published or written by another person, except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

[redacted]

Chandni Sengupta

Publications

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List of Abbreviations

ACTH - adrenocorticotropic hormone

CBG - corticosteroid binding globulins

CM - corticosterone metabolites

CRH - corticotrophin-releasing hormone

e.p.g - eggs per gram

EIA - Enzyme ImmunoAssay

F – Female

FCM - Faecal Cortisol Metabolites

FCM - faecal cortisol metabolites

fE_2 - faecal estradiol

FEC - Faecal Egg Count

fGC - faecal glucocorticoids

fGCM - faecal glucocorticoid metabolites

FGM - faecal glucocorticoid metabolites

fP_4 - faecal progesterone

fT - faecal testosterone

g - gram

GC - Glucocorticoid

GCM -glucocorticoid metabolites

HPA - Hypothalamic pituitary axis

M - Male

N.S.W - New South Wales

ng - nanogram

p.d - post defecation

RIA - radio immunoassay

S.E. – standard error

Tmax – maximum temperature

Tmin – minimum temperature

wt - weight

Abstract

Populations of bare-nosed wombats (*Vombatus ursinus*) are under threat across Australia and one of the major causes of their mortality is sarcoptic mange. Reasons for this high susceptibility are being investigated. On conducting a thorough search through literature, it was observed that although the current treatment practice can successfully restrict sarcoptic mange prevalence in small captive bare-nosed wombat populations, the same is not true for large free-ranging populations. Environmental factors such as drought, habitat degradation and anthropogenic interference can act as potential stress factors to exacerbate the effects of sarcoptic mange. During stress, the hypothalamic pituitary adrenal (HPA) axis is stimulated to release stress hormones (glucocorticoids - GC) that can influence immune function. Past research has suggested that chronic stress is associated with immunosuppression which can play a role in disease incidence. Additionally, a high prevalence of secondary endoparasitic infestations have been reported to be associated with sarcoptic mange incidence. To better comprehend the parameters that influence sarcoptic mange incidence in bare-nosed wombats, this study employed non-invasive procedures that enable evaluation of endoparasitic and stress load in these marsupials.

Non-invasive faecal sampling is ideal for large nocturnal marsupials like bare-nosed wombats. Validation of species-specific enzyme immunoassay (EIA) is necessary. This thesis aimed to optimise techniques suitable for quantifying faecal cortisol metabolites (FCM) (end products of HPA axis activation) in the voided excreta of bare-nosed wombats. One in-house and one commercial EIA was successfully validated to monitor the adrenocortical activity in these marsupials. Faecal samples collected from free-ranging wombats are usually <12 h old. Faecal hormone metabolites are vulnerable to bacterial activity and hence the knowledge of the decay rate of these metabolites in voided faeces is essential. This study determined the decay rate and baseline level of FCM in captive bare-nosed wombat faecal samples.

Faecal samples collected from free-ranging wombats located in five different locations in N.S.W, Australia were analysed to evaluate their endoparasite and stress load simultaneously. My research brought new

insights into the current parasitological and stress profiles of bare-nosed wombats as well as the current sarcoptic mange prevalence in these marsupials at the five study sites. This study demonstrated that an increase in FCM level among bare-nosed wombat populations could increase the probability of being infected with sarcoptic mange. The outcome of this study agrees with the corticosteroid-fitness hypothesis, which envisages that an increase in the levels of GC hormones can result in reduced fitness of organisms and hence can result in lower fecundity and higher risk of developing an infection. This can lower chances of future population survival. To the best of my knowledge, this is the first study that incorporates endoparasitic load, chronic stress and sarcoptic mange prevalence in bare-nosed wombat populations in N.S.W, Australia in both empirical and experimental approaches.

Chapter 1: Introduction

1.1 Background and Rationale

Mammals in Australia are under threat. Short and Smith (1994) reported that Australia have witnessed nearly half of all mammalian extinctions, while in a recent study by Woinarski et al. (2015) reported that more than one third of the mammals in Australia are endangered. Of the three extant wombat species, the northern hairy-nosed wombat (*Lasiorhinus krefftii*) is Critically Endangered (CR), the southern hairy-nosed wombat (*Lasiorhinus latifrons*) is Nearly Threatened (NT) and the bare-nosed wombat (*Vombatus ursinus*) is considered Least Concern (LC) (Taggart et al., 2016a, Taggart et al., 2016b, Woinarski and Burbidge, 2016). However, a decline in populations of bare-nosed wombat have been noted (Buchan and Goldney, 1998, Roger et al., 2007). Investigating the causes of this population decline and the associated reasons is essential. Simultaneously, conservation efforts are necessary to prevent populations from diminishing further.

Populations of bare-nosed wombats are under threat due to vehicle collisions, habitat fragmentation, bushfires, drought, predators and diseases (Roger et al., 2007, Triggs and Goldingay, 1996). Of all the threats to the survival of the bare-nosed wombat, the most debilitating and crippling is sarcoptic mange (Alasaad et al., 2011). Growing molecular evidence suggests that this disease is not native to Australia but suspected to be introduced during European settlement and since then the disease has spread and infested seven mammal species in Australia (Fraser et al., 2016, Pence and Ueckermann, 2002).

Although sarcoptic mange has made an impact worldwide (on humans, wild and domestic animals) (Alasaad et al., 2011, Pence and Ueckermann, 2002, Tompkins et al., 2015) little is known about the role of stress in its incidence. For example, physiological stress is known to cause negative consequences on the immune function, reproduction and behaviour of native wildlife species (Hing et al., 2016a, Hogan and Tribe, 2007, Narayan and Hero, 2014). Chronic stress or prolonged exposure to environmental

stressors could increase the incidence of infectious disease in wildlife as a direct health consequence (Hing et al., 2016b).

Secondary bacterial infections and high prevalence of gut parasites are often observed to be associated with sarcoptic mange infested animals (Skerratt, 2001, Balestrieri et al., 2006, Skerratt, 1998). There is an existing gap in scientific knowledge on the current parasitic load of the bare-nosed wombats infected with sarcoptic mange. Gaining knowledge about the role of chronic stress and the parasitic load of bare-nosed wombats with sarcoptic mange will help in designing future conservation measures for the marsupial.

This thesis explores the ecology of sarcoptic mange disease by determining if chronic stress and/or endoparasitic load increases the susceptibility to sarcoptic mange in bare-nosed wombats. The terms incidence and prevalence of diseases has been mentioned in this thesis. Prevalence is defined as the total number of individuals in a population who have a disease or health condition at a specific period of time, usually expressed as a percentage of the population (www.hsph.havard.edu, 2019). Incidence is defined as the number of individuals who develop a specific disease or experience a specific health-related event during a particular time period (such as a month or year) (www.hsph.havard.edu, 2019). Therefore, incidence is generally considered to convey information about the risk of contracting a disease whereas prevalence indicates how widespread is the disease within a population.

1.2 Bare-nosed wombats

1.2.1 Wombat as a marsupial species

Wombats are large fossorial nocturnal marsupials that inhabit parts of the Australian mainland and Tasmania (Triggs and Goldingay, 1996). These marsupials belong to the order Diprotodontia, superfamily Vombatidae (Fig 1.1) and share several similar characteristics with koalas (*Phascolarctos cinereus*), such as the number of chromosomes, backward facing pouch with one pair of teats, sperm morphology and rudimentary tails (Hughes, 1965, Martin and Hayman, 1967, Triggs, 2009). Hermsen (2015) has also reported a significant similarity in MHCII gene sequence identity between bare-nosed wombats and koalas. Another distinct characteristic of these marsupials is a pair of large procumbent rootless incisors

which grow unceasingly (Crompton et al., 2008, McIlroy, 2008). Being burrowing animals, the wombats are characterised by strong-clawed feet along with muscular legs and broad skull, short neck, robust pectoral and pelvic girdles.

At present, there are three extant species of wombats living in Australia. Of the three, two have hairy noses and narrowed nasal bones (Triggs, 2009). These marsupials are found in the northern and southern parts of Australia. These two species are known as northern hairy-nosed wombats and southern hairy-nosed wombats respectively. The third species of wombat has coarse short hair on its snout and is known as the bare-nosed wombat.

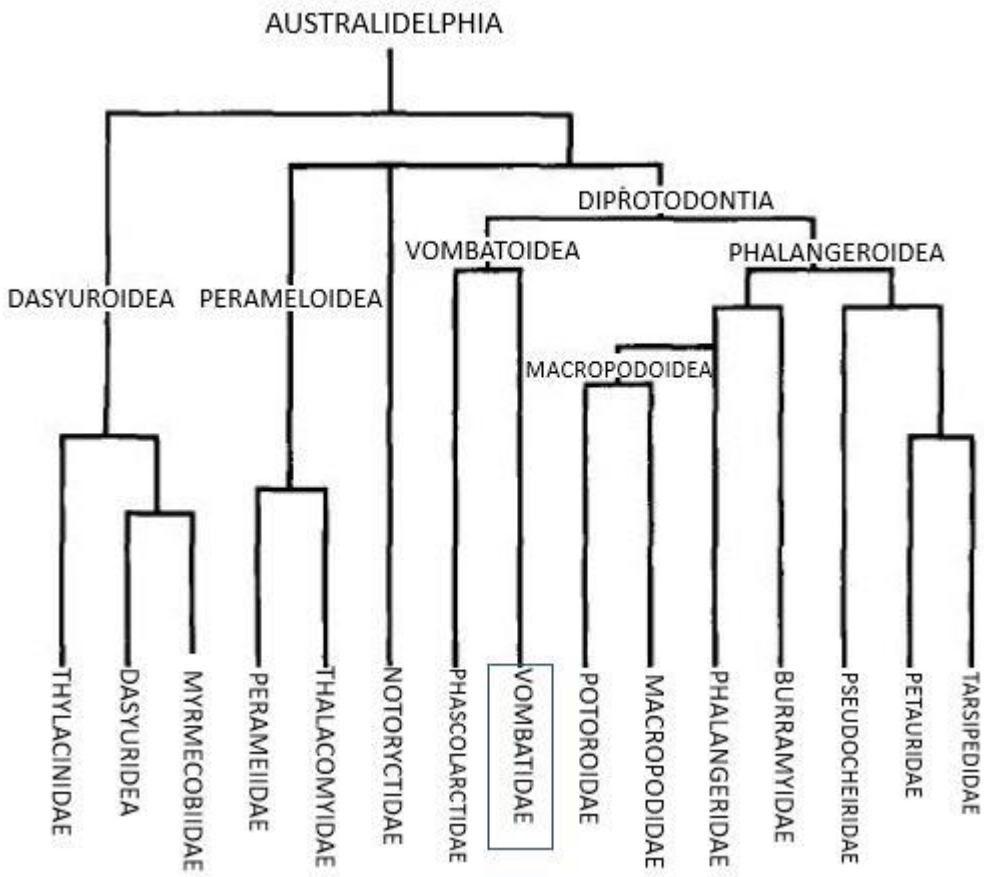

Figure 1.1: Cladogram showing relationship between different groups and subgroups of Australian marsupials, where our subgroup of interest has been highlighted in blue (Based on Archer (1984).

1.2.2 General biology

Bare-nosed wombats are covered with a smooth coat that varies from brown, grey, black and fawn (Triggs and Goldingay, 1996). Typically, a thickset body and small ears characterises the bare-nosed wombats (Triggs, 2009).On attainment of sexual maturity at two years of age they reproduce every two years (Triggs, 2009). Breeding peaks in the spring season but occurs throughout the year (McEwan, 2000). The gestation period lasts for about twenty to thirty days with the joeys staying in the pouch for five months (McIlroy, 1973). The young are weaned at twelve to fifteen months (Skerratt, 2001). The average lifespan of a free-ranging wild wombat is 15 years; however, wombats can live up to 20 – 30 years in captivity (WomSAT.org.au, 2017).

1.2.3 Habitat and burrow range

Wombats are large burrow living animals (Triggs, 2009). Wombats prefer burrows that are deep and thermally favourable (Shimmin et al., 2002). These animals have low energy necessities and therefore can endure harsh climatic conditions and exploit habitats which are low in produce (Barboza, 1993, Evans et al., 2003). The wombats use a strategy of minimal ranging behaviour and have physiological adaptations that maintain a low energy expenditure (Finlayson et al., 2005, Johnson, 1998).

Evans (2008) reported that bare-nosed wombats inhabit three vegetation types, namely eucalypt forest, woodland and pasture, with the eucalypt forest and pasture being their most preferred vegetation type. The average home range of bare-nosed wombats is 17.7 ha, while the average core area is 2.9 ha (Evans, 2008). As wombats are burrowing animals, they are considered important ecosystem engineers (Fleming et al., 2014, Old et al., 2018), with their burrow making habits helping soil turnover. Unique dentition and an efficient gastrointestinal system aid wombats in extracting nutrients from a high fibre diet as well as helping to conserve energy (Evans et al., 2006).

1.2.4 Distribution of bare-nosed wombats

Bare-nosed wombats are distributed in Victoria, New South Wales, the Australian Capital Territory, Tasmania and Flinders Island, the south-eastern part of South Australia and south-eastern part of

Queensland (McIlroy, 1995). Evidence suggests that the European settlement has marked a reduction in the range of bare-nosed wombats , predominantly in western Victoria, southern Queensland, and northern South Australia (Buchan and Goldney, 1998, McIlroy, 1995, Triggs and Goldingay, 1988) (Fig 1.2). Recent estimates of bare-nosed wombat population densities provide more accurate numbers of wombats in the wild (Hermsen, 2015, Hunter, 2011, WomSAT.org.au, 2017).

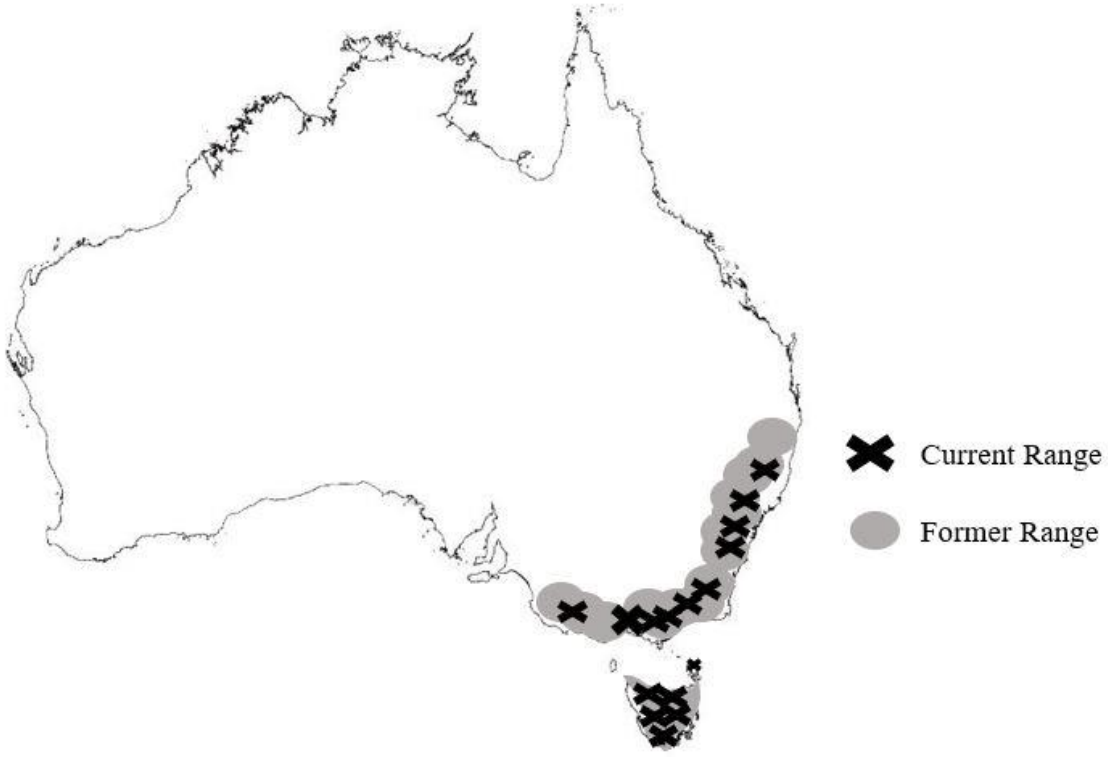

Figure 1.2: Current bare-nosed wombat distribution. (Map drawn by C. Sengupta with information from Taggart et al. (2016b))

1.3 Factors responsible for wombat population decline

Wombats have decreased in number for the past century because of historic hunting for the fur trade and loss of native grasslands (F.R., 1903, Triggs and Goldingay, 1996). This century, wombats in the wild encounter climatic changes, human interference, intra- and inter-species competition for food, vehicle collisions and habitat loss and destruction. (Roger et al., 2011, Skerratt, 1998, Triggs, 2009, Woolnough and Johnson, 2000, Hermsen, 2015, Martin et al., 1998). Furthermore, the attitude of past governments

and land owners were reported by Temby (1998) and Matthews et al. (2011). These attitudes are still a concern (Marks, 1998, Matthews et al., 2011) since culling of wombats is allowed even today in some parts of the country (Temby, 1998, DELWP, 2018). Apart from these threats, diseases are one of the main concerns that can have deleterious effects on the wombat population.

In this thesis, three key threats that may be responsible for the decline of bare-nosed wombats have been investigated – sarcoptic mange, gastrointestinal helminths and chronic stress. These are discussed below.

1.3.1 Sarcoptic mange in bare-nosed wombats

Bare-nosed wombats are found to be most affected by sarcoptic mange amongst all other affected animals in Australia – koala, agile wallaby (*Macropus agilis*), dingo (*Canis familiaris dingo*), southern hairy-nosed wombats, European red fox (*Vulpes vulpes*), one-humped camel (*Camelus dromedaries*), dog (*Canis familiaris*), pig (*Sus scrofa*) and horse (*Equus caballus*) (Barbet, 2014, Brown et al., 2004, Davies et al., 1991, Fleming et al., 2001, McLelland and Youl, 2005, Obendorf, 1983, Pence and Ueckermann, 2002, Ruykys et al., 2013, Saunders et al., 1995). Wombats may become infected with mites from other infected animals that are sharing the same burrows, as has been observed by Gerasimov (1958) in foxes. The disease is caused by an astigmatic mite named *Sarcoptes scabiei*. Male mites range from 213-285 μm in length and 162-210 μm in breadth, whereas female mites are larger in size and range from 300-504 μm in length and 230-420 μm in width (Arlian, 1989).

The first mite found to infect bare-nosed wombats was discovered by Latreille (1817). Adult mites tunnel into the epidermis of the skin and lay eggs inside these tunnels. Subcutaneous tunnelling causes severe irritation. Severe itching sensation is a characteristic feature of hypersensitivity to the disease in animals and humans (Arlian, 1989, Brugess, 1994, Skerratt, 2001). Heavily infected wombats spend a large time scratching to alleviate irritation (Simpson et al., 2016). However, scratching results in higher energy demand (Hartley and English, 2005), and as a result nocturnal wombats are found foraging during the day to satisfy their increased energy requirement (Borchard et al., 2012, Fain, 1978, Mounsey et al., 2008,

Ruykys et al., 2009). Wombats severely infested with *S. scabiei* become emaciated, lack hair with their skin forming a dry crust composed of keratin. The crusted keratin is usually full of mites, debris from the mites, bacteria and cellular remains (Presidente, 1982, Skerratt et al., 1998, Skerratt, 2001, Sweatman, 1971). Presence of crust around the eyes and ears of these animals often leads to them being blind and deaf (Hartley and English, 2005, Pence and Ueckermann, 2002). Secondary bacterial infections in the crusted keratin is common and can lead to septicaemia and other internal infections (Skerratt, 1998). Inability to eat and secondary bacterial infections usually result in the death of the wombat.

Figure 1.3: A bare-nosed wombat severely infested with sarcoptic mange. (Image: C. Sengupta. Wolgan Valley; August 2018).

1.3.2 Helminths in bare-nosed wombats

The helminth parasites of bare-nosed wombats are typical of grazing mammals (Table 1.1). Some strongyloids present are also found in macropodid hosts, and attributable to the host switching phenomena (Beveridge and Spratt, 1996) (Fig 1.3). Common liver fluke (*Fasciola hepatica*) is the only known trematode to infect the bare-nosed wombats (Beveridge and Spratt, 1996, Smales, 1998). *F. hepatica* is

present in the liver of bare-nosed wombats and can lead to fibrosis in the bile ducts, as well as extensive hepatic fibrosis. Small scale liver fluke infection is usually observed in wombats, indicating they are fairly resistant to the disease (Spratt and Presidente, 1981).

The cestode parasites of bare-nosed wombats are dominated by *Phascolotaenia* and *Paramoniezia*, inhabiting the small intestine and bile ducts of wombats respectively. These parasites are apparently non-pathogenic and Beveridge and Spratt (1996) suggested that it is probable that *Phascolotaenia* is derived from *Paramoniezia.* Spratt et al. (1991) has described *E. granulosus* from captive bare-nosed wombats, however Doube (1981) was unable to detect *E. granulosus* infection in other surveys, suggesting that bare-nosed wombats are not suitable hosts and that the infection could only occur in unusual circumstances in captivity. Furthermore, larval stages of *Taenia hydatigena* are found to cause granuloma in the liver of bare-nosed wombats (Presidente, 1982) but due to an absence of peritoneal cysticercus formation, are considered unsuitable hosts (Table 1.1). Additionally, *Anoplotaenia dasyuri* is also considered an atypical parasite for bare-nosed wombats, since Beveridge et al. (1975) was unable to induce the infection in this species. *Programotaenia festiva*, occasionally found in the bile ducts, has been reported to cause hypertrophy and proliferation of mucus glands in bare-nosed wombats (Presidente and Beveridge, 1978).

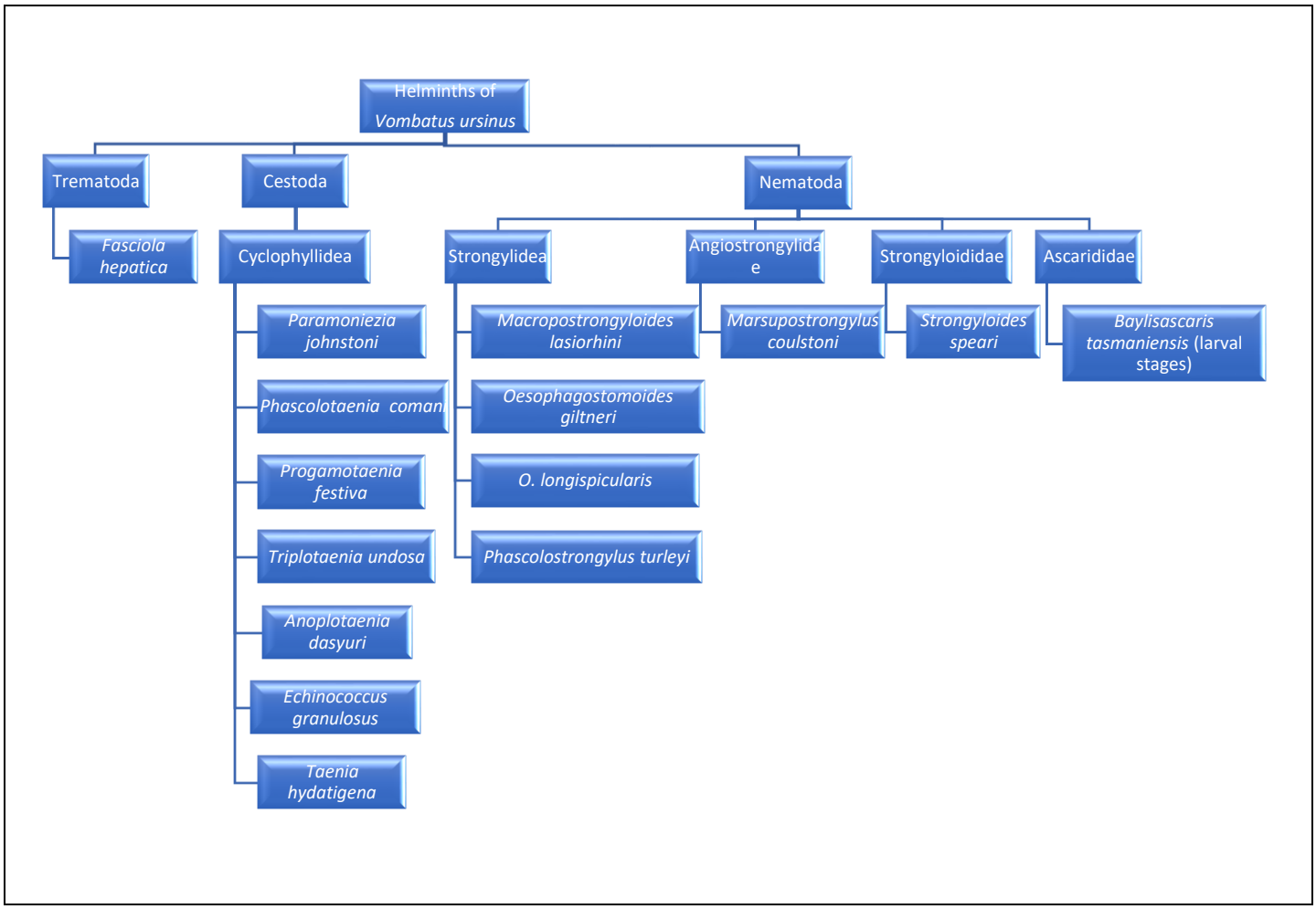

Figure 1.4: Current diversity of helminth parasites of bare-nosed wombats. [Data source Beveridge and Spratt, 1996; Smales, 1998]

Macropostrongyloides, Oesophagostomoides and *Phascolostronglus* are inhabitants of the colon of bare-nosed wombats and the predominate nematode fauna. The three genera comprise six species and may occur in large numbers (6000-6500 nematodes per wombat colon) (Beveridge, 1978). *Strongyloides spearei* found in the small intestine mucosa has been associated with a mild enteritis in bare-nosed wombats (Presidente, 1982). Although Skerratt (1998) found no pathological response in bare-nosed wombats due to the presence of these nematode parasites (Table 1.1), significant impact due to the presence of *Strongyloides* sp. eggs in the gut have been documented in captive kangaroos (*Macropus* sp.) (Winter, 1958), free-ranging eastern grey kangaroos (*M. giganteus*) (Arundel et al., 1990), agile wallabies (*M.*

agilis) (Speare et al., 1983), and spectacled hare-wallabies (*Largorchestus conspicillatus*) (Speare et al., 1983). Arundel et al. (1977) reported a high increase in mortality rates of eastern grey kangaroos, wallaroos (*M. robustus*) and red kangaroos (*M. rufus*) because of the infection caused by *Strongyloides* sp.

Larval stages of the ascarid parasite of the Tasmanian devil (*Sarcophilus harrisi*), called *Baylisascaris tasmaniensis* has been identified in the granulomatous lesions of several organs and mesenteries of bare-nosed wombats (Munday and Gregory, 1974). However, bare-nosed wombats are only considered an intermediate host for this parasite (Sprent et al., 1973). *Marsupostrongylus coulstoni* have been found in the lungs of bare-nosed wombats, but no systemic disease has been reported to be associated with the infection (Smales, 1998) (Table 1.1).

An immunological response is typically the outcome of an interaction between ectoparasites and endoparasite. *S. scabiei* infection elicits variable degrees of both T-helper 1 and T-helper 2 immune responses (Arlian and Morgan, 2000, Bornstein et al., 2001, Lalli et al., 2004) whereas helminths induce a T-helper 2 immune response (Pritchard et al., 1997). However, T-helper 1 and T-helper 2 immunological responses are mutually exclusive, which would mean that the immune response could be biased, and thus favour the parasite involved in the other T-helper response (Cox, 2001).

Sarcoptic mange and other infections not only activate the bare-nosed wombat's immune system but also stimulates a complex endocrine pathway (Skerratt, 2001). Chronic diseases like sarcoptic mange can disrupt homeostasis (O'Connor et al., 2000). In an attempt to regain homeostasis (an individual's steady state), the body activates a negative feedback system of hormones whose end products are glucocorticoids (O'Connor et al., 2000), and the endocrine response is highly conserved in vertebrates (Sheriff et al., 2011).

Table 1.1: Pathogenicity of helminths in bare-nosed wombats

Helminth name	Pathogenicity in bare-nosed wombats	References
Trematode		
Fasciola hepatica	usually harmless; at a larger scale can cause fibrosis is bile ducts	Beveridge and Spratt, 1996; Smales, 1998; Spratt and Presidente 1981
Cestode		
Phascolotaenia comani	non pathogenic	Beveridge and Spratt, 1996
Paramoniezia johnstoni	non pathogenic	Beveridge and Spratt, 1996
Progamotaenia festiva	hypertrophy and proliferation of mucous gland of bile ducts	Presidente and Beveridge 1978
E. granulosus	infection found only in captivity	Spratt, Beveridge and Walter 1991
Taenia hydatigena	granuloma in liver; absence of peritoneal cysts; not suitable hosts	Presidente 1982
Aniplotaenia dasyuri	non pathogenic	Beveridge et al. 1975
Nematode		
Macropostrongyloides sp.	potentially pathogenic; may feed on blood cells	Beveridge and Mawson 1978
Oesophagostomoides sp.	non pathogenic	Beveridge 1978
Phascolostrongylus sp.	non pathogenic	Beveridge 1978
Marsupostrongylus coulstoni	found in lungs; no systemic disease reported	Smales 1998
Strongyloides speari	mild enteritis; usually non-pathogenic	Presidente 1982; Skerratt 1995
Baylisascaris tasmaniensis	granulomatous lesions of several organs and mesenteries	Munday and Gregory 1974

1.3.3. Chronic stress in bare-nosed wombats

Wikelski and Cooke (2006),in their review, reported that an individual's aptitude to cope against noxious stimulus in the environment is usually estimated by the stress level of the individual. All factors responsible for challenging homeostasis is referred to as a stressor. The concept of homeostasis or sense of constancy of "milieu intérieu" was developed in the 19th and 20th century with the works of Bernard and Cannon (McEwen and Stellar, 1993). However, even under normal circumstances, physiological functions (such as blood pressure, heart rate, neural and endocrinal activity) are not static but are constantly altering in response to the environment across an operating range (McEwen and Stellar, 1993). Sterling and Eyer (1988) defined the ability of the body to respond to these environmental challenges and adapt its internal systems to the new steady state as allostasis. McEwen and Wingfield (2007) explicated allostatic load as the stress response of an individual in the presence of repeated and predictable environmental challenge. On any occasion when an unpredictable stressor forces an organism into an event of "allostatic overload", the animal modulates its behaviour and physiology to enable it to survive (Wikelski and Cooke, 2006).

Wild animals are exposed to habitat destruction, climate change, competition for food and diseases, consequently they exhibit a complex biological reaction in response to these stressors. This reaction is termed, the physiological stress response, and can be further classified into acute and chronic stress responses (Dantzer et al., 2014, Sheriff et al., 2011). When experiencing a short time stressor stress hormones like adrenaline and glucocorticoids (GC) rise well above baseline levels within minutes to provoke a flight-fight response, which includes physiological and immunological changes such as an increase in heart rate, respiration rate and increased glucose metabolism (Dantzer et al., 2014, Sheriff et al., 2011). However, these changes are reversible, and the level of stress hormones returns to pre-stressor concentrations after a short time. This kind of stress response is characteristic of an acute stress response. During chronic stress, i.e., prolonged exposure to a stressor such as climate change or starvation, the raised levels of stress hormones return to baseline concentrations after a longer period of time (Dantzer et al.,

2014, Sheriff et al., 2011). Chronic exposure to elevated stress hormones can have harmful effects such as reduced reproduction, muscle degeneration, and immunosuppression (Sapolsky, 2002). Therefore, during chronic stress, the GC levels remain above baseline levels and can have direct negative effects on the fitness of the individual. Researchers refer to this phenomenon in terms of the cortisol (cort)-fitness hypothesis, and it has been critically reviewed by Bonier et al. (2009). At elevated levels of GC, the body tries to survive the challenge at the cost of important physiological functions like reproduction, which results in reduced fitness. Since this variation in baseline GC is a heritable component, this feature will be reflected in the genetics of the individual. Thus, organisms with a more diverse genetic makeup can endure stress more readily than individuals with lower genetic diversity. Therefore, Bonier et al. (2009) suggested that there is a negative relationship between fitness and baseline GCs, i.e., organisms with higher levels of genetic fitness will have lower levels of baseline GC in the presence of a chronic stressor and vice versa.

During an event of allostatic overload, GC is released by adrenal glands via the hypothalamic-pituitary-adrenal (HPA) axis in vertebrates. GCs are a class of adrenal hormones that are present in systemic blood circulation (Westphal, 1969, Bush, 1957). The behaviour, development and physiology of an animal are regulated by these adrenal hormones (Denver, 2009). During a stress event, the first phase of the HPA axis, the hypothalamus is stimulated and releases corticotrophin-releasing hormone (CRH). Adrenocorticotropic hormone (ACTH) is released when CRH reaches the anterior pituitary gland via the hypothalamo-hypophyseal portal system. The released ACTH travels through blood to the adrenal cortex and stimulates the production of GCs. The GC hormones bind with the GC receptors present in the pituitary, hypothalamus and hippocampus to initiate a negative feedback loop of the HPA axis which in turn maintains homeostasis (de Kloet et al., 1998).

The GCs released in blood are bound to carrier proteins called the corticosteroid binding globulins (CBG) (Westphal, 1969, Bush, 1957) and this CBG-GC complex moves through the bloodstream to reach the target tissue cells (Sheriff et al., 2011, Daughaday, 1959). About eighty to ninety percent of GCs remain bound to CBG in blood and only a small portion are free or are unbound GC molecules. These free GC molecules are considered as “biologically active” and are responsible for bringing about the stress

response, i.e., inhibition or activation of the physiological system in response to a stressor (Tait et al., 1964, Westphal, 1969). The free GC molecules get metabolized in the liver and are excreted as conjugates of glucuronides and sulphides via faeces and/or urine of an animal (Palme et al., 2005, Daughaday, 1959, Bongiovanni and Eberlein, 1955).

Bare-nosed wombats are exposed to different kinds of stressors such as drought, bushfire, diseases and habitat destruction (Hermsen, 2015, Skerratt, 1998, Triggs and Goldingay, 1996, Wells and Pridmore, 1998). Although several studies have investigated the stress physiology in southern hairy nosed wombats (Descovich et al., 2012a, Descovich et al., 2012b, Du et al., 2018, Hogan et al., 2011, Du et al., 2017), there is a scarcity of published data on the stress physiology of bare-nosed wombats. Chronic stress has been suggested to increase the potential risk of disease susceptibility (Table 1.2). Furthermore, stressors can exacerbate the effects of a disease, since stress can reduce the immunity of an individual (Sapolsky, 2002). Martin et al. (1998) noted the prevalence of sarcoptic mange increased with decreased food availability, such as during drought and winter, or due to habitat degradation. In addition, Zumpt and Ledger (1973) reported underlying disease to be one of the factors that can predispose an animal to develop severe and chronic sarcoptic mange. The relationship between sarcoptic mange outbreak and drought has been suspected in southern hairy-nosed wombats (Ruykys et al., 2009). However, the role of chronic stress in sarcoptic mange incidence in bare-nosed wombats remains understudied.

Table 1.2: Studies suggesting chronic stress can increase disease susceptibility.

Animal	Disease Susceptibility	Reference
Gracilinanus agilis	*Eimeria spp* Infection	Hernandez et al. (2018)
Petrochelidon pyrrhonota	Hematophagous swallow bug (*Oeciacus vicarius*)	Raouf et al. (2006)
Sciurus griseus	Notoedric mange	Vander Haegen et al. (2018)
Cardinalis cardinalis	West Nile Virus	Owen et al. (2012)
Litoria wilcoxii	Chytridiomycosis	Kindermann et al. (2012)
Migratory birds	pathogenic avian influenza	Weber and Stilianakis (2007)
Captive marsupials	Toxoplasmosis	Thompson et al. (2010)
Phascolarctos cinereus	*Chlamydia* infection	Brearley et al. (2013)
Pteropus spp.	Hendra virus (HeV infection)	Plowright et al. (2008)

1.4 Non-Invasive Field Sampling

Bare-nosed wombats are large animals with adults weighing 26 kg – 35 kg (McIlroy, 2008). Collecting blood samples from wombats would require physically restraining and capturing the animals. Capturing and restraining wombats would cause distress, be detrimental to their health, and as a consequence would alter their behavioural and endocrinal state (Whitten et al., 1998). Voided excreta can easily be collected from free-living and captive animals. Excreta contain metabolites of GC that are analogous to serum GC profiles (Whitten et al., 1998). Excreted GC metabolites does not reflect a single point in time but rather the average values combined over time (Schwarzenberger, 2007). Enzyme-immunoassays (EIA) of the hormone metabolites extracted from the faeces or urine thus reflect the "true baseline" levels of GC in blood instead of the GC extracted from the blood of the animal, which is usually referred to as the "nominal baseline" level (Sheriff et al., 2011). However, validation of EIAs are necessary for each species because of the species-specific differences in hormone metabolism (Goymann, 2012, Schwarzenberger, 2007, Touma and Palme, 2005).

Validation involves analytical, biological and physiological validation of the EIA technique (Touma and Palme, 2005). Analytical validation constitutes examining the sample preservation and sample stability in addition to the extraction procedure involved and the antibody used. Physiological validation often involves injecting the subject with appropriate ACTH or dexamethasone doses to check whether these doses can induce a change in the subject's plasma GC level and whether the change is reflected also in the excreted GC metabolites. Biologically validating a technique requires exposing an animal to a stressor (human interaction, translocation, capture, food deprivation) and examining whether this exposure has caused a change in the excreted GC metabolite level. Once the EIAs are validated, non-invasive endocrine assessments can be conducted to accurately monitor the stress physiology of animals (Schwarzenberger, 2007). Validated non-invasive endocrine assessment can act as a powerful tool of conservation physiology (Wikelski and Cooke, 2006, Palme, 2019).

Wikelski and Cooke (2006) defined conservation physiology as the study of physiological responses of plants and animals to anthropogenic alteration of the environment (for example endocrine and immune responses to environmental changes) which can predict a potential population decline. Conservation practitioners and environmental ecologists not only identify and lessen potential threats to wildlife but also reinstate natural ecosystems by employing non-invasive techniques. For example, exhaled breath condensate was collected to monitor stress load in belugas (*Delphinapterus leucas*) (Thompson et al., 2014). Faecal glucocorticoid metabolites were quantified to determine the stress load in captive koalas, free-ranging bandicoots (*Perameles nasuta* and *Isoodon obesulus)*, captive bilbies (*Macrotis lagotis*) and endangered woylies (*Bettongia penicillata*) (Dowle et al., 2013, Evans et al., 2013, Hing et al., 2017, Narayan et al., 2013). Longitudinal studies on faecal or urinary glucocorticoid metabolites in conjunction with other factors such as reproduction and behaviour were conducted to comprehend stress physiology in tigers (*Panthera tigris tigris)* and leopards *(Panthera pardus fusca),* southern hairy-nosed wombats, and adult male stony creek frogs (*Litoria wilcoxii*) (Du et al., 2018, Hogan et al., 2011, Kindermann et al., 2012, Paris et al., 2002). Faecal progesterone metabolite concentrations were studied in endangered tree kangaroos (*Dendrolagus matschiei*) to determine the temporal features of their oestrus cycle (North and

Harder, 2008). Non-invasive detection methods of *Chlamydia* disease were developed utilising faecal samples in vulnerable koala populations (Cristescu et al., 2019). Hermsen (2015) also conducted non-invasive genetic studies in bare-nosed wombats to investigate their increased sarcoptic mange susceptibility. In this study, non-invasive sampling was used to determine the sarcoptic mange prevalence, stress level and endoparasitic load in free-ranging and captive bare-nosed wombats.

1.5 Aims

Martin et al. (1998) reported sarcoptic mange as one of the principal causes of mortality in bare-nosed wombats. Although clinical aspects of the disease have been explored, little effort has focused on determining the reasons of this high susceptibility of wombats to sarcoptic mange. No literature has been published in the past exploring the effects of chronic stress and endoparasitic loads on bare-nosed wombats affected with or without sarcoptic mange.

The primary aim of the literature review was to identify the vital gaps in literature focussing on sarcoptic mange in bare-nosed wombats, which is presented in Chapter 2.

The first experimental aim of this study was to conduct optimisation experiments. Non-invasive sampling is ideal to monitor stress physiology in bare-nosed wombats. However, faecal samples collected from free-ranging wombats are usually <12 h old. Therefore, it was necessary to determine the rate of decay of faecal cortisol metabolites in voided faeces of bare-nosed wombats (Chapter 3). Additionally, species-specific EIA validations are required prior to any investigation of the stress physiology (Touma and Palme, 2005). Validation of EIAs to accurately monitor adrenocortical activity of these marsupials was conducted (Chapter 3). Baseline stress levels of bare-nosed wombats was determined in this study.

The second experimental aim was to correlate the degree of incidence of chronic stress, gut parasites and sarcoptic mange in bare-nosed wombats. Identification of the stressors and recognition of the stress-disease relationship can help future wildlife ecologists successfully manage affected animals (Hing et al., 2014). Therefore, analysing the relationship between stress and incidence of sarcoptic mange is essential for the health and welfare of bare-nosed wombats. In order to better understand the link between chronic stress, gut parasites (helminths) and sarcoptic mange incidence in bare-nosed wombats, the current stress load, endoparasitic load and sarcoptic mange prevalence was determined non-invasively at five different locations in N.S.W, Australia (Chapter 4). The degree of correlation between chronic stress, endoparasitic load and sarcoptic mange prevalence was thereafter established.

Chapter 5 discusses the key findings of this research in addition to the recommendations and future implications. Overall, the outcomes of the study have provided new insights into the factors that influence disease incidence in wild wombat populations and the information can be used to support conservation management programs.

1.6 Thesis outline

The thesis is separated into five chapters (including this introduction and a discussion), focussing on sarcoptic mange disease in bare-nosed wombats. Three of the following five chapters are written as publications and differ in format according to the journal to which they were submitted. The following four chapters are:

Chapter 2: Sarcoptic mange in wombats: A review and future directions

Chapter 3: Testing the environmental decay of faecal cortisol metabolites in bare-nosed wombats (*Vombatus ursinus*)

Chapter 4: Evaluating the role of stress and helminth load in sarcoptic mange incidence in free-ranging and captive bare-nosed wombats (*Vombatus ursinus*)

Chapter 5: General discussions, recommendations and future directions

1.7 References

Alasaad, S., Walton, S., Rossi, L., Bornstein, S., Abu-Madi, M., Soriguer, R. C., Fitzgerald, S., Zhu, X.-Q., Zimmermann, W. & Ugbomoiko, U. S. 2011. Sarcoptes-world molecular network (Sarcoptes-WMN): integrating research on scabies. *International Journal of Infectious Diseases,* **15,** e294-e297

Archer, M. 1984. The Australian marsupial radiation. In'Vertebrate Zoogeography and Evolution in Australasia'.(Eds M. Archer and G. Clayton.) pp. 633-808. Hesperian Press: Perth.

Arlian, L. G. 1989. Biology, host relations, and epidemiology of *Sarcoptes scabiei*. *Annual review of entomology*, **34,** 139-159

Arlian, L. G. & Morgan, M. S. 2000. Serum antibody to *Sarcoptes scabiei* and house dust mite prior to and during infestation with *S. scabiei*. *Veterinary parasitology,* **90,** 315-326

Arundel, J., Barker, I. & Beveridge, I. 1977. Diseases of marsupials. *The biology of marsupials*. Springer.

Arundel, J., Dempster, K., Harrigan, K. & Black, R. 1990. Epidemiological observations on the helminth parasites of *Macropus giganteus* Shaw in Victoria. *Wildlife Research,* **17,** 39-51

Balestrieri, A., Remonti, L., Ferrari, N., Ferrari, A., Valvo, T. L., Robetto, S. & Orusa, R. 2006. Sarcoptic mange in wild carnivores and its co-occurrence with parasitic helminths in the Western Italian Alps. *European Journal of Wildlife Research,* **52,** 196-201

Barbet, J. L. 2014. Chapter 59 - Ectoparasites of Horses A2 - Sellon, Debra C. *In:* Long, M. T. (ed.) *Equine Infectious Diseases (Second Edition)*. St. Louis: W.B. Saunders.

Barboza, P. S. 1993. Digestive strategies of the wombats: feed intake, fiber digestion, and digesta passage in two grazing marsupials with hindgut fermentation. *Physiological Zoology*, **66,** 983-999 https://doi.org/10.1086/physzool.66.6.30163750

Beveridge, I. 1978. A Taxonomic Revision of the Genera Phascolostrongylus Canavan, and Oesophagostomoides Schwartz (Nematoda: Trichonematidae) From Wombats. *Australian Journal of Zoology*, **26,** 585-602

Beveridge, I. & Mawson, P. 1978. A Taxonomic Revision of the Genera Macropostrongyloides Yamaguti and Paramacropostrongylus Johnston & Mawson (Nematoda: Trichonematidae) From

Australian Marsupials. *Australian Journal of Zoology,* **26,** 763-787 https://doi.org/10.1071/ZO9780763

Beveridge, I., Rickard, M., Gregory, G. & Munday, B. 1975. Studies on *Anoplotaenia dasyuri* Beddard, 1911 (Cestoda: Taeniidae), a parasite of the Tasmanian devil: Observations on the egg and metacestode. *International Journal for Parasitology,* **5,** 257-267

Beveridge, I. & Spratt, D. 1996. The helminth fauna of Australasian marsupials: origins and evolutionary biology. *Advances in Parasitology (United Kingdom),*

Bongiovanni, A. M. & Eberlein, W. R. 1955. Determination, Recovery, Identification and Renal Clearance of Conjugated Adrenal Corticoids in Human Peripheral Blood. *Proceedings of the Society for Experimental Biology and Medicine,* **89,** 281-285 10.3181/00379727-89-21785

Bonier, F., Martin, P. R., Moore, I. T. & Wingfield, J. C. 2009. Do baseline glucocorticoids predict fitness? *Trends in Ecology & Evolution,* **24,** 634-642

Borchard, P., Eldridge, D. J. & Wright, I. A. 2012. Sarcoptes mange (*Sarcoptes scabiei*) increases diurnal activity of bare-nosed wombats (*Vombatus ursinus*) in an agricultural riparian environment. *Mammalian Biology-Zeitschrift für Säugetierkunde,* **77,** 244-248

Bornstein, S., Mörner, T. & Samuel, W. M. 2001. *Sarcoptes scabiei* and sarcoptic mange. *Parasitic Diseases of Wild Mammals, Second Edition,* 107-119

Brearley, G., Rhodes, J., Bradley, A., Baxter, G., Seabrook, L., Lunney, D., Liu, Y. & Mcalpine, C. 2013. Wildlife disease prevalence in human-modified landscapes. *Biological Reviews,* **88,** 427-442 https://doi.org/10.1111/brv.12009

Brown, J., Walker, S. & Steinman, K. 2004. Endocrine manual for the reproductive assessment of domestic and non-domestic species, Endocrine Research Laboratory, Conservation and Research Center, National Zoological Park, Smithsonian Institution, Virginia.

Brugess, I. 1994. *Sarcoptes scabiei* and scabies. *Advances in Parasitology,* **33,** 235-292

Buchan, A. & Goldney, D. C. 1998. The common wombat *Vombatus ursinus* in a fragmented landscape. *Wombats'.(Eds RT Wells and PA Pridmore.) pp,* 251-261

Bush, I. The physicochemical state of cortisol in blood. Ciba Foundation Symposium-Hormones in Blood (Colloquia on Endocrinology), 1957. Wiley Online Library, 263-285.

Cox, F. 2001. Concomitant infections, parasites and immune responses. *Parasitology,* **122,** S23-S38 https://doi.org/10.1017/S003118200001698X

Cristescu, R. H., Miller, R. L., Schultz, A. J., Hulse, L., Jaccoud, D., Johnston, S., Hanger, J., Booth, R. & Frère, C. H. 2019. Developing non-invasive methodologies to assess koala population health through detecting *Chlamydia* from scats. *Molecular Ecology Resources,* doi:10.1111/1755-0998.12999

Crompton, A. W., Lieberman, D. E., Owerkowicz, T., Baudinette, R. V. & Skinner, J. 2008. Motor control of masticatory movements in the Southern hairy-nosed wombat (*Lasiorhinus latifrons*). *Primate craniofacial function and biology***,** 83-111

Dantzer, B., Fletcher, Q. E., Boonstra, R. & Sheriff, M. J. 2014. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? *Conservation Physiology,* **2**

Daughaday, W. H. 1959. Steroid-protein interactions. *Physiological reviews,* **39,** 885-902

Davies, Moore & Pointon 1991. Seasonality of sarcoptic mange in pigs in South Australia. *Australian Veterinary Journal,* **68,** 390-392 10.1111/j.1751-0813.1991.tb03105.x

De Kloet, E., Vreugdenhil, E., Oitzl, M. & JoëLs, M. 1998. Brain Corticosteroid Receptor Balance in Health and Disease. *Endocrine Reviews,* **19,** 269-301 10.1210/edrv.19.3.0331

Delwp. 2018. *Authorities to Control Wildlife* [Online]. Available: https://www.wildlife.vic.gov.au/__data/assets/pdf_file/0016/113029/ATCW-Data_annual-data-2009-2017.pdf [Accessed 18 February 2019].

Denver, R. J. 2009. Structural and functional evolution of vertebrate neuroendocrine stress systems. *Annals of the New York Academy of Sciences,* **1163,** 1-16

Descovich, K. A., Lisle, A. T., Johnston, S., Keeley, T. & Phillips, C. J. 2012a. Intrasample variation and the effect of storage delay on faecal metabolite concentrations in the southern hairy-nosed wombat (*Lasiorhinus latifrons*). *Australian mammalogy,* **34,** 217-222

Descovich, K. A., Lisle, A. T., Johnston, S., Nicolson, V. & Phillips, C. J. 2012b. Differential responses of captive southern hairy-nosed wombats (*Lasiorhinus latifrons*) to the presence of faeces from different species and male and female conspecifics. *Applied Animal Behaviour Science,* **138,** 110-117

Doube, L. Diseases of wombats. Wildlife Diseases of the Pacific Basin and Other Countries. Proceedings of the 4 th International Conference of the Wildlife Disease Association. Sydney, 1981. 63-75.

Dowle, M., Webster, K. N. & Deane, E. 2013. Faecal glucocorticoid metabolite concentrations in the free-ranging bandicoots (*Perameles nasuta* and *Isoodon obesulus*) of northern Sydney. *Australian Mammalogy,* **35,** 1-7

Du, Z., Johnston, S. D., Janssen, T., Phillips, C. J. C., Lisle, A. & Keeley, T. 2018. Behavioral and endocrine responses to season and social dynamics of captive male southern hairy-nosed wombats (*Lasiorhinus latifrons*). *General and Comparative Endocrinology,* **261,** 40-50 https://doi.org/10.1016/j.ygcen.2018.01.021

Du, Z., Keeley, T., Janssen, T., Nicolson, V. & Johnston, S. 2017. Measurement of testosterone and cortisol metabolites and luteinising hormone in captive southern hairy-nosed wombat (*Lasiorhinus latifrons*) urine. *General and comparative endocrinology,* **250,** 70-79

Evans, M., Green, B. & Newgrain, K. 2003. The field energetics and water fluxes of free-living wombats (Marsupialia: Vombatidae). *Oecologia,* **137,** 171-180 https://doi.org/10.1007/s00442-003-1322-4

Evans, M. C. 2008. Home range, burrow-use and activity patterns in common wombats (*Vombatus ursinus*). *Wildlife Research,* **35,** 455-462

Evans, M. C., Macgregor, C. & Jarman, P. J. 2006. Diet and feeding selectivity of common wombats. *Wildlife Research,* **33,** 321-330

Evans, N., Narayan, E. J. & Hero, J.-M. 2013. Effects of natural weathering conditions on faecal cortisol metabolite measurements in the greater bilby (*Macrotis lagotis*). *Australian journal of zoology,* **61,** 351-356

F.R. 1903. The Australian Fur Trade. *The Brisbane Courier (Qld. : 1864 - 1933)* Sat 03 1903.

Fain, A. 1978. Epidemiological problems of scabies. *International Journal of Dermatology,* **17,** 20-30

Finlayson, G., Shimmin, G., Temple-Smith, P., Handasyde, K. & Taggart, D. 2005. Burrow use and ranging behaviour of the southern hairy-nosed wombat (*Lasiorhinus latifrons*) in the Murraylands, South Australia. *Journal of Zoology,* **265,** 189-200 https://doi.org/10.1017/S095283690400620X

Fleming, P., Corbett, L., Harden, R. & Thompson, P. 2001. Managing the Impact of Dingoes and Other Wild Dogs.,(Australian Government Publishing: Canberra.).

Fleming, P. A., Anderson, H., Prendergast, A. S., Bretz, M. R., Valentine, L. E. & Hardy, G. E. 2014. Is the loss of Australian digging mammals contributing to a deterioration in ecosystem function? *Mammal Review,* **44,** 94-108

Fraser, T. A., Charleston, M., Martin, A., Polkinghorne, A. & Carver, S. 2016. The emergence of sarcoptic mange in Australian wildlife: an unresolved debate. *Parasites & vectors,* **9,** 316

Gerasimov, Y. 1958. Mange in wild foxes. *Translation of Russian game reports. Canadian Department of Northern Affairs National Resources, Ottawa***,** 1-5

Goymann, W. 2012. On the use of non-invasive hormone research in uncontrolled, natural environments: the problem with sex, diet, metabolic rate and the individual. *Methods in Ecology and Evolution,* **3,** 757-765

Hartley, M. & English, A. 2005. *Sarcoptes scabei var. wombat*i infection in the common wombat (*Vombatus ursinus*). *European Journal of Wildlife Research,* **51,** 117-121

Hermsen, E. 2015. Analysis of non-invasive sampling techniques to determine sarcoptic mange distribution and MHC diversity in the bare-nosed wombat (*Vombatus ursinus*). Bachelor of Science, Western Sydney University.

Hernandez, S., Strona, A., Leiner, N., Suzán, G. & Romano, M. 2018. Seasonal changes of faecal cortisol metabolite levels in *Gracilinanus agilis* (Didelphimorphia: Didelphidae) and its association to life histories variables and parasite loads. *Conservation Physiology,* **6,** coy21 https://doi.org/10.1093/conphys/coy021

Hing, S., Currie, A., Broomfield, S., Keatley, S., Jones, K., Thompson, R. A., Narayan, E. & Godfrey, S. S. 2016a. Host stress physiology and Trypanosoma haemoparasite infection influence innate immunity in the woylie (*Bettongia penicillata*). *Comparative immunology, microbiology and infectious diseases,* **46,** 32-39

Hing, S., Jones, K. L., Rafferty, C., Thompson, R. A., Narayan, E. J. & Godfrey, S. S. 2017. Wildlife in the line of fire: evaluating the stress physiology of a critically endangered Australian marsupial after bushfire. *Australian Journal of Zoology,* **64,** 385-389

Hing, S., Narayan, E., Thompson, R. A. & Godfrey, S. 2014. A review of factors influencing the stress response in Australian marsupials. *Conservation Physiology,* **2,** cou027

Hing, S., Narayan, E. J., Thompson, R. A. & Godfrey, S. S. 2016b. The relationship between physiological stress and wildlife disease: consequences for health and conservation. *Wildlife Research,* **43,** 51-60

Hogan, L. A., Johnston, S. D., Lisle, A. T., Keeley, T., Wong, P., Nicolson, V., Horsup, A. B., Janssen, T. & Phillips, C. J. C. 2011. Behavioural and physiological responses of captive wombats (*Lasiorhinus latifrons*) to regular handling by humans. *Applied Animal Behaviour Science,* **134,** 217-228 10.1016/j.applanim.2011.07.010

Hogan, L. A. & Tribe, A. 2007. Prevalence and cause of stereotypic behaviour in common wombats (*Vombatus ursinus*) residing in Australian zoos. *Applied Animal Behaviour Science,* **105,** 180-191 https://doi.org/10.1016/j.applanim.2006.06.006

Hughes, R. 1965. Comparative morphology of spermatozoa from five marsupial families. *Australian Journal of Zoology*, **13,** 533-544 https://doi.org/10.1071/ZO9650533

Hunter, N. 2011. The effect of sarcoptic mange on the ecology of the common wombat *(Vombatus ursinus)*. Bachelor of Science (Honours) thesis, University of Western Sydney.

Johnson, C. 1998. The evolutionary ecology of wombats. *In:* Rt, W. & Pa, P. (eds.) *Wombats*. Chipping, Norton: Surrey Beatty & Sons.

Kindermann, C., Narayan, E. J. & Hero, J.-M. 2012. Urinary corticosterone metabolites and chytridiomycosis disease prevalence in a free-living population of male Stony Creek frogs (*Litoria wilcoxii*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* **162,** 171-176 https://doi.org/10.1016/j.cbpa.2012.02.018

Lalli, P. N., Morgan, M. S. & Arlian, L. G. 2004. Skewed Th1/Th2 immune response to *Sarcoptes scabiei*. *Journal of Parasitology*, **90,** 711-714

Latreille, M. 1817. *Nouveau Dictionnaire d'Histoire Naturelle,* **21,** 222-223

Marks, C. A. 1998. Review of the humaneness of destruction techniques used on the common wombat *Vombatus ursinus* in Victoria. *Wombats'.(Eds RT Wells and PA Pridmore.) pp***,** 287-297

Martin, P. & Hayman, D. 1967. Quantitative comparisons between the karyotypes of Australian marsupials from three different superfamilies. *Chromosoma,* **20,** 290-310

Martin, R., Handasyde, K. & Skerratt, L. 1998. Current distribution of sarcoptic mange in wombats. *Australian veterinary journal,* **76,** 411-414

Matthews, A., Lunney, D., Crowther, M., Bryant, J. & Shannon, I. 2011. The distribution and community perceptions of the common wombat in New South Wales. *National Wombat Conference.*

Mcewan, S. R.-M. 2000. Monitoring reproduction in the common wombat (*Vombatus ursinus*) and Tasmanian devil (*Sarcophilus harrisii*) through faecal steroid analysis.

Mcewen, B. S. & Stellar, E. 1993. Stress and the individual: mechanisms leading to disease. *Archives of internal medicine,* **153,** 2093-2101

Mcewen, B. S. & Wingfield, J. C. 2007. Allostasis and allostatic load. *In:* In G. Fink, E. (ed.) Encyclopedia of Stress, Ed 2. ed. Academic Press, New York.

Mcilroy, J. 1995. Common wombat. *The mammals of Australia. Reed Books, Chatswood, New South Wales,* 204-205

Mcilroy, J. 2008. The mammals of Australia. *In:* Van Dyck, S. & Strahan, R. (eds.) 3rd ed. ed. Chatswood, New South Wales: Reeds Book: New Holland Publishers.

Mcilroy, J. C. 1973. Aspects of the Ecology of the Common Wombat, *Vombatus Ursinus* (Shaw, 1800), Australian National University.

Mclelland, D. & Youl, J. 2005. Sarcoptic mange in agile wallabies (*Macropus agilis*) in the Northern Territory. *Australian veterinary journal,* **83,** 744-745

Mounsey, K. E., Holt, D. C., Mccarthy, J., Currie, B. J. & Walton, S. F. 2008. Scabies: molecular perspectives and therapeutic implications in the face of emerging drug resistance. *Future microbiology,* **3,** 57-66

Munday, B. & Gregory, G. 1974. Demonstration of larval forms of Baylisascaris tasmaniensis in the wombat (Vombatus ursinus). *Journal of wildlife diseases,* **10,** 241-242

Narayan, E. J. & Hero, J.-M. 2014. Acute Thermal Stressor Increases Glucocorticoid Response but Minimizes Testosterone and Locomotor Performance in the Cane Toad (*Rhinella marina*). *PLOS ONE,* **9,** e92090 10.1371/journal.pone.0092090

Narayan, E. J., Webster, K., Nicolson, V., Mucci, A. & Hero, J.-M. 2013. Non-invasive evaluation of physiological stress in an iconic Australian marsupial: the koala (*Phascolarctos cinereus*). *General and Comparative Endocrinology,* **187,** 39-47

North, L. A. & Harder, J. D. 2008. Characterization of the estrous cycle and assessment of reproductive status in Matschie's tree kangaroo (*Dendrolagus matschiei*) with fecal progestin profiles. *General and comparative endocrinology,* **156,** 173-180 https://doi.org/10.1016/j.ygcen.2007.12.007

O'connor, T. M., O'halloran, D. J. & Shanahan, F. 2000. The stress response and the hypothalamic-pituitary-adrenal axis: from molecule to melancholia. *QJM: An International Journal of Medicine,* **93,** 323-333 10.1093/qjmed/93.6.323

Obendorf, D. L. 1983. Causes of mortality and morbidity of wild koalas, *Phascolarctos cinereus* (Goldfuss), in Victoria, Australia. *Journal of wildlife diseases,* **19,** 123-131

Old, J. M., Hunter, N. E. & Wolfenden, J. 2018. Who utilises bare-nosed wombat burrows? *Australian Zoologist,* **39,** 409-413 10.7882/AZ.2018.006

Owen, J. C., Nakamura, A., Coon, C. A. & Martin, L. B. 2012. The effect of exogenous corticosterone on West Nile virus infection in Northern Cardinals (*Cardinalis cardinalis*). *Veterinary Research,* **43,** 34 10.1186/1297-9716-43-34

Palme, R. 2019. Non-invasive measurement of glucocorticoids: Advances and problems. *Physiology & behavior,* **199,** 229-243 https://doi.org/10.1016/j.physbeh.2018.11.021

Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S. & Möstl, E. 2005. Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. *Annals of the New York Academy of Sciences,* **1040,** 162-171

Paris, M., White, A., Reiss, A., West, M. & Schwarzenberger, F. 2002. Faecal progesterone metabolites and behavioural observations for the non-invasive assessment of oestrous cycles in the common wombat (*Vombatus ursinus*) and the southern hairy-nosed wombat (*Lasiorhinus latifrons*). *Animal Reproduction Science,* **72,** 245-257 https://doi.org/10.1016/S0378-4320(02)00088-X

Pence, D. B. & Ueckermann, E. 2002. Sarcoptic mange in wildlife. *Rev. sci. tech. Off. int. Epiz.,* **21,** 385-398

Plowright, R. K., Field, H. E., Smith, C., Divljan, A., Palmer, C., Tabor, G., Daszak, P. & Foley, J. E. 2008. Reproduction and nutritional stress are risk factors for Hendra virus infection in little red flying foxes (*Pteropus scapulatus*). *Proceedings of the Royal Society of London B: Biological Sciences,* **275,** 861-869

Presidente, P. 1982. Common wombat Vombatus ursinus: maintenance in captivity, blood values, infectious and parasitic diseases. *The Management of Australian Mammals in Captivity. Zoological Parks Board of Victoria: Melbourne***,** 133-143

Presidente, P. & Beveridge, I. 1978. Cholangitis Associated With Species Of Progamotaenia (Cestoda: Anoplocephalidae) In The Bile Ducts Of marsupials. *Journal of Wildlife Diseases,* **14,** 371-377 10.7589/0090-3558-14.3.371

Pritchard, D., Hewitt, C. & Moqbel, R. 1997. The relationship between immunological responsiveness controlled by T-helper 2 lymphocytes and infections with parasitic helminths. *Parasitology,* **115,** 33-44

Raouf, S. A., Smith, L. C., Brown, M. B., Wingfield, J. C. & Brown, C. R. 2006. Glucocorticoid hormone levels increase with group size and parasite load in cliff swallows. *Animal Behaviour,* **71,** 39-48 https://doi.org/10.1016/j.anbehav.2005.03.027

Roger, E., Laffan, S. W. & Ramp, D. 2007. Habitat selection by the common wombat (*Vombatus ursinus*) in disturbed environments: implications for the conservation of a 'common'species. *Biological Conservation,* **137,** 437-449

Roger, E., Laffan, S. W. & Ramp, D. 2011. Road impacts a tipping point for wildlife populations in threatened landscapes. *Population Ecology,* **53,** 215-227

Ruykys, L., Breed, B., Schultz, D. & Taggart, D. 2013. Effects and treatment of sarcoptic mange in southern hairy-nosed wombats (*Lasiorhinus latifrons*). *Journal of wildlife diseases,* **49,** 312-320

Ruykys, L., Taggart, D. A., Breed, W. G. & Schultz, D. 2009. Sarcoptic mange in southern hairy-nosed wombats (*Lasiorhinus latifrons*): distribution and prevalence in the Murraylands of South Australia. *Australian journal of zoology,* **57,** 129-138

Sapolsky, R. M. 2002. Endocrinology of the stress-response. *Behavioral endocrinology., 2nd ed.,*

Saunders, G., Coman, B., Kinnear, J. & Braysher, M. 1995. Managing vertebrate pests: foxes, Australian Government Publ. Service.

Schwarzenberger, F. 2007. The many uses of non-invasive faecal steroid monitoring in zoo and wildlife species. *International Zoo Yearbook,* **41,** 52-74 https://doi.org/10.1111/j.1748-1090.2007.00017.x

Sheriff, M. J., Dantzer, B., Delehanty, B., Palme, R. & Boonstra, R. 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia,* **166,** 869-887

Shimmin, G. A., Skinner, J. & Baudinette, R. V. 2002. The warren architecture and environment of the southern hairy-nosed wombat (*Lasiorhinus latifrons*). *Journal of Zoology,* **258,** 469-477 https://doi.org/10.1017/S0952836902001620

Short, J. & Smith, A. 1994. Mammal Decline and Recovery in Australia. *Journal of Mammalogy,* **75,** 288-297 10.2307/1382547

Simpson, K., Johnson, C. N. & Carver, S. 2016. Sarcoptes scabiei: the mange mite with mighty effects on the common wombat (*Vombatus ursinus*). *PloS one,* **11,** e0149749

Skerratt, L., Martin, R. & Handasyde, K. 1998. Sarcoptic mange in wombats. *Australian veterinary journal.* Victoria,Australia.

Skerratt, L. F. 1998. Diseases and parasites of the common wombat *Vombatus ursinus* in the Healesville area of Victoria. Surrey Beatty & Sons.

Skerratt, L. F. 2001. Sarcoptic mange in the common wombat, *Vombatus ursinus* (Shaw, 1800).

Smales, L. 1998. Helminth parasites of wombats. *Wombats***,** 312-316

Speare, R., Beveridge, I. & Johnson, P. 1983. Parasites of the Agile Wallaby, *Macropus Agilis* (Marsupialia). *Wildlife Research,* **10,** 89-96 https://doi.org/10.1071/WR9830089

Spratt, D., Beveridge, I. & Walter, E. 1991. A catalogue of Australasian monotremes and marsupials and their recorded helminth parasites. *Records of the South Australian Museum, Monograph Series,* **1,** 105

Spratt, D. M. & Presidente, P. J. 1981. Prevalence of *Fasciola hepatica* infection in native mammals in southeastern Australia. *Aust J Exp Biol Med Sci,* **59,** 713

Sprent, J. F. A., Lamina, J. & A., M. 1973. Observations on the migratory behaviour and development of *Baylisascaris tasmaniensis*. *Parasitology,* **67,** 67-83

Sterling, P. & Eyer, J. 1988. Allostasis: a new paradigm to explain arousal pathology. In 'Handbook of Life Stress, Cognition and Health'.(Eds K. Fisher and J. Reason.) pp. 629–649. Wiley: New York.

Sweatman 1971. Mites and pentasomes. *In:* Davis Jw, A. R., Editors (ed.). Ames: Iowa State University Press.

Taggart, D., Martin, R. & Horsup, A. 2016a. *Lasiorhinus krefftii. The IUCN Red List of Threatened Species 2016: e.T11343A21959050* [Online]. Available: http://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T11343A21959050.en [Accessed 9 June 2017].

Taggart, D., Martin, R. & Menkhorst, P. 2016b. *Vombatus ursinus. The IUCN Red List of Threatened Species 2016: e.T40556A21958985* [Online]. Available: http://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T40556A21958985.en [Accessed 9 June 2017].

Tait, J., Burstein, S., Pincus, G., Thimann, K. & Astwood, E. 1964. The hormones. *Eds. G. Pincus, KV Thimann and EB,* 441

Temby, I. 1998. The law and wombats in Australia. *Wombats'.(Eds RT Wells and PA Pridmore.) pp,* 305-311

Thompson, L. A., Spoon, T. R., Goertz, C. E., Hobbs, R. C. & Romano, T. A. 2014. Blow collection as a non-invasive method for measuring cortisol in the beluga (*Delphinapterus leucas*). *PLoS One,* **9,** e114062 https://doi.org/10.1371/journal.pone.0114062

Thompson, R. C. A., Lymbery, A. J. & Smith, A. 2010. Parasites, emerging disease and wildlife conservation. *International Journal for Parasitology,* **40,** 1163-1170 http://doi.org/10.1016/j.ijpara.2010.04.009

Tompkins, D. M., Carver, S., Jones, M. E., Krkošek, M. & Skerratt, L. F. 2015. Emerging infectious diseases of wildlife: a critical perspective. *Trends in Parasitology,* **31,** 149-159

Touma, C. & Palme, R. 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. *Annals of the New York Academy of Sciences,* **1046,** 54-74

Triggs, B. 2009. Wombats, Collingwood, Vic., CSIRO PUBLISHING.

Triggs, B. & Goldingay, R. 1988. The wombat : common wombats in Australia, [Kensington, N.S.W.], New South Wales University Press.

Triggs, B. & Goldingay, R. 1996. The wombat: common wombats in Australia, University of New South Wales Press Australia.

Vander Haegen, W. M., Orth, G. R., Johnston, A. N. & Linders, M. J. 2018. Endemic diseases affect population dynamics of tree squirrels in contrasting landscapes. *Journal of Wildlife Management,* **82,** 328-343 https://doi.org/10.1002/jwmg.21383

Weber, T. P. & Stilianakis, N. I. 2007. Ecologic immunology of avian influenza (H5N1) in migratory birds. *Emerging infectious diseases,* **13,** 1139 10.3201/eid1308.070319

Wells, R. & Pridmore, P. 1998. Wombats, University of Minnesota Press.

Westphal, U. 1969. [41] Assay and properties of corticosteroid-binding globulin and other steroid-binding serum proteins. *Methods in Enzymology,* **15,** 761-796 10.1016/S0076-6879(69)15043-0

Whitten, L., Brockman, D. & Stavisky, R. 1998. Recent Advances in Noninvasive Techniques to Monitor Hormone-Behavior Interactions. *American Journal of Physical Anthropology,* **Suppl 27,** 1-23 10.1002/(SICI)1096-8644(1998)107:27+3.0.CO;2-H

Wikelski, M. & Cooke, S. J. 2006. Conservation physiology. *Trends in Ecology & Evolution,* **21,** 38-46

Winter, H. 1958. Gastric strongyloidosis in kangaroos. *Australian Veterinary Journal,* **34,** 118-120

Woinarski, J. & Burbidge, A. A. 2016. *Lasiorhinus latifrons. The IUCN Red List of Threatened Species 2016: e.T40555A21959203*

[Online]. Available: http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T40555A21959203.en [Accessed 9 June 2017].

Woinarski, J. C. Z., Burbidge, A. A. & Harrison, P. L. 2015. Ongoing unraveling of a continental fauna: Decline and extinction of Australian mammals since European settlement. *Proceedings of the National Academy of Sciences,* **112,** 4531 10.1073/pnas.1417301112

Womsat.Org.Au. 2017. *WomSAT.org.au* [Online]. NSW, Australia. Available: https://womsat.org.au/womsat/pagecontent.aspx?page=wombat_contacts [Accessed].

Woolnough, A. P. & Johnson, C. N. 2000. Assessment of the potential for competition between two sympatric herbivores-the northern hairy-nosed wombat, *Lasiorhinus krefftii*, and the eastern grey kangaroo, *Macropus giganteus*. *Wildlife Research,* **27,** 301-308

Www.Hsph.Havard.Edu. 2019. *Prevalence and Incidence Defined* [Online]. Available: https://www.hsph.harvard.edu/obesity-prevention-source/prevalence-incidence/ [Accessed 16-August-2019 2019].

Zumpt, F. & Ledger, J. 1973. Present epidemiological problems of sarcoptic mange in wild and domestic animal. *South African Journal of Wildlife Research,* **3,** 119-120

Chapter 2: Sarcoptic mange in wombats – A review and future research directions

2.1 Chapter Outline:

Sarcoptic mange is the major cause of mortality in bare-nosed wombats. This chapter provides a detailed literature review on the degree and nature of sarcoptic mange infestation in wombats and provides a critical assessment on current treatment practices. The review presents insights into reasons that may influence the high susceptibility of wombats to sarcoptic mange. The review also discusses current and potential research directions.

The manuscript is jointly authored, where Associate Professor is the primary and the corresponding author. She designed and conducted the burrow flap experiment mentioned in the paper, supervised the development of the manuscript and provided editorial feedback on manuscript drafts. I am the second author and I did research on past literature and drafted the manuscript. Dr. Edward Narayan and Jack Wolfenden provided feedback on manuscript drafts.

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Sarcoptic mange in wombats—A review and future research directions

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Summary

Sarcoptic mange is caused by the mite *Sarcoptes scabiei* and has recently been recognized as an emerging infectious disease of wildlife worldwide. The mite is one of the main causes of population decline in southern hairy-nosed (*Lasiorhinus latifrons*) and bare-nosed wombats (*Vombatus ursinus*). This review focuses on Sarcoptes scabiei infestations in wombats and provides insights into why the disease may be so prevalent in wombats. Current treatment practices and trials conducted in the field to reduce the incidence of sarcoptic mange in wombats are described and critically reviewed. Current and potential future avenues of research are discussed.

KEYWORDS
disease, ectoparasite, immune system, one health Sarcoptes scabiei, stress

2.2 | INTRODUCTION

Wombats are native grazers and "ecosystem engineers" (Fleming et al., 2014; Jones, Lawton, & Shachak, 1997). They affect rates of soil turnover and impact soil nutrition (Kinlaw, 1999), and their burrow building activity provides habitat for other species. Loss of fossorial mammals such as wombats can lead to decreases in water infiltration and may increase the chances of extensive drought and large-scale tree collapse (Fleming et al., 2014). Changes in soil properties are direct consequences of reduced fossorial mammals (Bretz, 2012) and can result in changes in the floristic community (Dickman, 2006). Loss of plant dispersal and recruitment and distribution of mycorrhizae may take longer to become apparent (Fleming et al., 2014). Wombats are, therefore, important contributors to ecosystem health and to ensure adequate levels of biodiversity are maintained.

Currently, there are three species of extant wombat, all native to Australia. Two of these wombat species are members of the Lasiorhinus genus. The southern hairy-nosed wombat (*L. latifrons*) is restricted to small fragmented areas, mostly in South Australia and is listed as "Vulnerable" (Woinarski & Burbidge, 2016). The northern hairy-nosed wombat (*L. krefftii*) is "Critically Endangered" (Taggart, Martin, & Horsup, 2016a) and restricted to two feral proof protected areas in Queensland (Johnson & Gordon, 1995).

The third species of extant wombat, the bare-nosed wombat (*Vombatus ursinus*), formerly known as the common wombat, is listed as "Least Concern" (Taggart, Martin, & Menkhorst, 2016b). At present, they are distributed in south-eastern South Australia, Victoria, New South Wales, south-eastern Queensland, Tasmania and Flinders Island (McIlroy, 1995). However, despite having this listing, like many other species, evidence suggests that there has been a reduction in bare-nosed wombat population since the 1700s (McIlroy, 1995; Triggs, 1998).

The wombat population was once widespread on the Australian mainland and Tasmania (Triggs, 1998). European settlement marked the beginning of land clearing and building of modern infrastructure, resulting in habitat destruction (McIlroy, 1973). Further, Temby (1998) has documented the attitude of landholders and past governments towards wombats and emphasized that wombats were considered vermin on pastoral lands. Some of these attitudes remain (Marks, 1998), and in some areas, culling of these native burrowers is still allowed (Temby, 1998). Death from vehicle collision has also impacted bare-nosed wombats. Roger, Bino, and Ramp (2012) stated 13.6% of wombats living in protected forest areas in NSW suffer road fatalities. Male wombats having larger home ranges compared to females tend to be more often killed (McIlroy, 1973; Skerratt, 1998). Skerratt (1998) has stated most wombats killed by road vehicles were usually healthy and free of mange. A range of other wombat parasites and diseases having been reported, including helminths (Skerratt, 1998; Smales, 1998) and toxoplasmosis (Donahoe et al., 2015); however, sarcoptic mange is the major threat to wombat survival.

2.3 | SARCOPTIC MANGE AND SARCOPTICS CABIEI

Sarcoptic mange occurs worldwide and is caused by an astigmatid ectoparasite, *Sarcoptes scabiei*, and has an extremely diverse host range (Pence & Ueckermann, 2002). The mite feeds off skin cells and serum as it burrows into the epidermal and dermal layers of its host, causing irritation, inflammation, hyperkeratosis, alopecia, pruritus, dermatitis and lesions that are typically coupled with pneumonia and secondary infections (Amer et al., 2014; Pence & Ueckermann, 2002).

Sarcoptic mange is zoonotic, infesting humans (scabies or Norwegian mange) and domestic animals (e.g., dogs), and imposes heavy welfare and economic burdens on production animal industries (e.g., cattle, pigs, goats, camelids) (Daszak, Cunningham, & Hyatt, 2000; Mounsey et al., 2015). For example, significant financial impacts have occurred in the pork industry due to sarcoptic mange (Damriyasa, Failing, Volmer, Zahner, & Bauer, 2004). Large-scale infestation of the mite reduces production efficiency in both breeding and fattening pigs (Davies, 1995). Pigs with skin lesions associated with mite infestations have been reported in Spain (Gutierrez, de Vigo, Castella, Munoz, & Ferrer, 1996), Germany (Damriyasa et al., 2004), USA (Davies, 1995) and Tanzania (Kambarage, Msolla, & Falmer-Hansen, 1990).

The recent resurgence and emergence of *Sarcoptes scabiei* on a global scale have led to its classification as an important emerging infectious disease of wildlife (Tompkins, Carver, Jones, Krkosek, & Skerratt, 2015), particularly owing to its host range expansion in Australia and North America (Alasaad et al., 2012). *Sarcoptes scabiei* has previously been reported in wildlife species worldwide including European red foxes (*Vulpes vulpes*) in the south-eastern United States (Little, Davidson, Howerth, Rakich, & Nettles, 1998) and Sweden (Danell & Hornfeldt,€ 1987; Lindstrom et al., 1994), coyotes (*Canis latrans*) in Texas (Pence & Windberg, 1994), pet rabbits (*Oryctolagus cuniculus*) in Israel (Eshar, 2010), camels (*Camelus dromedarius*) in Pakistan (Zahid et al., 2015) and Egypt (Kotb & Abdel-Rady, 2015), and African buffalo (*Syncerus caffer*) in Zambia (Munang'andu et al., 2010). In Australia, sarcoptic mange is widespread, affecting at least ten mammal species. The affected introduced mammals include the European red fox (Saunders, Coman, Kinnear, & Braysher, 1995), dog (*Canis familiaris*) (Fleming, Corbett, Harden, & Thompson, 2001), pig (*Sus scrofa*) (Davies, Moore, & Pointon, 1991), horse (*Equus caballus*) (Barbet, 2014) and one-humped camel (*Camelus dromedaries*) (Brown, 2004), whilst the affected wildlife species include the koala (*Phascolarctos cinereus*) (Obendorf, 1983; Pence & Ueckermann, 2002), agile wallaby (*Macropus agilis*) (McLelland & Youl, 2005), dingo (*Canis familiaris dingo*) (Fleming et al., 2001), and bare-nosed and southern hairy-nosed wombats (Ruykys, Breed, Schultz, & Taggart, 2013; Skerratt, 2001).

Wildlife is believed to be more susceptible to sarcoptic mange compared to humans and domestic species, due to an observed reduction in resistance to infection, environmental stress factors, such as harsh winters and drought, high host density, and environmental attributes favouring the growth and transmission of the parasite (Martin, Handasyde, & Skerratt, 1998). With the increasing threat of climate change, sarcoptic mange is suspected to increase its host range, and subsequently, global diversification of *Sarcoptes scabiei* will further impact humans, domestic animals and wildlife. Its range expansion into new locations and ability to infest new hosts has already resulted in high morbidity and mortality in some wildlife species (Pence & Ueckermann, 2002).

Womersley (1953) was the first to describe *Sarcoptes scabiei*; however, Fain (1978) and Arlian (1989) provided a more detailed description. *Sarcoptes scabiei* is oval with a ventrally flattened and dorsally convex tortoise-like body (Figure 2.1). It has stout dorsal setae, numerous cuticular spines and transversely ridged cuticular striations, with males smaller (213–285 μm long by 162–210 μm wide) than females (300–504 μm long by 230–420 μm wide) (Arlian, 1989; Fain, 1978). The first two legs of *Sarcoptes scabiei* females have tarsi, which have a single claw and a short pedunculate sucker. The epimera of leg I is joined apically, and the tarsus of legs I and II has stalked empodia that terminate in a pad. The first two legs of males, how-ever, have tarsal pedunculate suckers and paired bifurcated claws. The legs III and IV of females are short with claws and suckers but end in a pair of very long setae, with the genitalia located between these two legs. In males, the size of leg III is half the size of leg IV. Leg III bares a tarsus that ends in a short stout claw, whereas on leg IV there are two only setae and two lanceolate spines. The tibiotarsus of leg IV bears a stalked empodium that ends in a broad pad (Arlian, 1989; Womersley, 1953).

The life cycle of *Sarcoptes scabiei* comprises five stages and includes egg (Figure 2.2), larval, protonymphal instar, tritonymphal instar and adult (Arlian, Vyszenski-Moher, & Pole, 1989; Fain, 1978). The mature females lay 1–3 eggs per day in the epidermis of their host (Arlian, 1989). Its lifespan is 30–60 days and takes

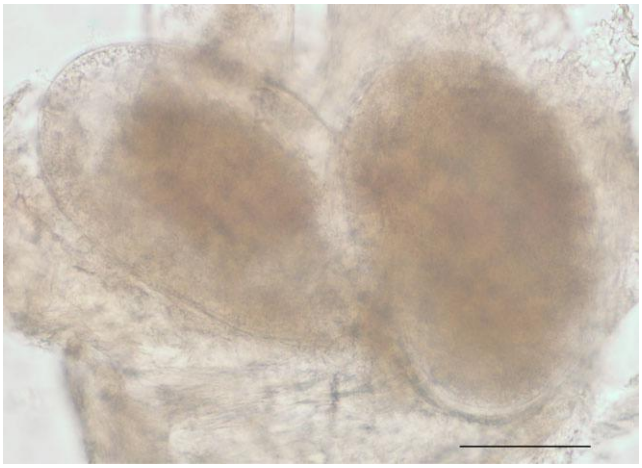

FIGURE 2.1 Photomicrograph of *Sarcoptes scabiei* mite eggs at 109 magnification. Mite eggs were obtained from the skin scrapings of a severely mange infested bare-nosed wombat (*Vombatus ursinus*). Scale bar 50 μm

approximately half its lifespan to reach maturity (Alexander, 1984). Skerratt (2001) reported that the ratio of male to female *Sarcoptes scabiei* collected from wombats was 2:1, and suggested males are capable of mating with more than one female and that the lifespan of males was less than that of females. Skerratt (2001) also observed lower numbers of nymphs compared to numbers of females and may suggest that they leave their host early in search of new hosts. These findings, however, differ to those of Castro et al. (2016) that described the larvae, nymph and adult sex ratios of Sarcoptes scabiei mites on Iberian ibex (*Capra pyrenaica*) and found more adult females than males (5.18:1) and that overall most mites were in the larval life cycle stage, rather than nymphs or adults. Given it is expected that there are likely to be more female mites than male mites, to provide increased mite reproductive rates, the wombat mite ratios may be due to the experiments being conducted in captivity, the length of time the study was conducted, or other factors such as season, host sex, age, body condition or weight, as described in Castro et al. (2016).

Host specificity of *Sarcoptes spp*. has been a topic of debate for some time (Bornstein, Morner, & Samuel, 2001; Daszak et al., 2000). Fain (1968) stated the genus Sarcoptes contained only one valid, but variable species, and in a subsequent publication, Fain (1978) failed to identify any morphological differences between populations of mites. More recently, this genus has been broadly classified according to the host species it infests, for example *Sarcoptes scabiei var. hominis* and *Sarcoptes scabiei var. canis* (Alasaad et al., 2011). Berrilli, D'Amelio, and Rossi (2002) have indicated that there is no epidemiological relationship between sarcoptic mange foci, and the success rate of experimental cross-infection is very low (Arlian et al., 1989);

F I G U R E 2.2 Photomicrograph of *Sarcoptes scabiei* adult mite at 109 magnification. The adult mite was obtained from the skin scrapings of a severely infested bare-nosed wombat (*Vombatus ursinus*). Scale bar 250 μm

however, *Sarcoptes scabiei var. vulpes/canis* is known to infest both dogs and other canids along with felids (Bornstein, Zakrisson, & Thebo, 1995). Furthermore, *Sarcoptes scabiei var. vulpes/canis* can also infest humans (Bornstein et al., 1995), despite it usually being limited to certain topographic areas (Alasaad et al., 2011).

The variety of *Sarcoptes scabiei* found on wombats, named *wombati* by Railliet (1895), was formally described by Womersley (1953). Humans, dogs and koalas are known to get infected with the mite on coming into contact with mite-infested wombats; however, limited knowledge is available about the host specificity of *Sarcoptes scabiei var. wombati.* (Arundel, Barker, & Beveridge, 1977; Barker, 1974; Gray, 1937; Railliet, 1895; Skerratt, 2001).

Knowledge of host specificity of *Sarcoptes scabiei* has advanced in the last few decades with improvements and further developments in molecular biology. Molecular studies based on short fragments of mitochondrial or ribosomal DNA spacer regions failed to identify *Sarcoptes scabiei* populations to the host species level and geographical localities (Alasaad et al., 2008; Fain, 1978; Gu & Yang, 2008; Skerratt et al., 2002; Zahler, Essig, Gothe, & Rinder, 1999). However, studies on a central fragment of the 16S gene and the complete cytochrome c oxidase subunit I gene (COI) in combination with microsatellite markers have provided some support for a genetic differentiation between *Sarcoptes scabiei* varieties (Walton et al., 2004). These genetic markers demonstrated significant relationships between *Sarcoptes scabiei* mitochondrial DNA (mtDNA) haplotypes and microsatellite allele frequencies, and host species and geographical locations (Alasaad et al., 2008; Walton et al., 1999, 2004). A further study revealed that if the mangy host's body was divided into areas, the subpopulations of mites infesting these different areas were genetically diverse (Alasaad et al., 2008).

In her review, Fraser, Charleston, Martin, Polkinghorne, and Carver (2016) emphasized that the 16S rRNA fragment, COI DNA fragment of the mitochondria and microsatellites are important genetic markers in establishing host-specific differences. In contrast, 12S rRNA has been found to be relatively unreliable when considering building a phylogenetic relationship between host species and *Sarcoptes scabiei* populations (Alasaad et al., 2008; Walton et al., 2004). The phylogenetic tree built by Fraser et al. (2016) with 16S rRNA sequences of *Sarcoptes spp*. from across the world suggested that there are two distinct clades of *Sarcoptes scabiei.* One clade consisted of human and animal *Sarcoptes scabiei* of European origin, whilst the other clade consisted of *Sarcoptes scabiei* infesting humans and animals from the remainder of the world. On the other hand, the same tree built using COI sequences obtained from the same global locations indicated that the clades separated as human and animal clades and did not indicate any geographical separation (Fraser et al., 2016).

2.4 | WOMBATS ARCOPTIC MANGE

Of the seven native animal species in Australia infested by *Sarcoptes scabiei*, wombats are the most highly impacted, with significant population declines having been observed in New South Wales and Tasmania (Fraser et al., 2016). The earliest records of mange on an Australian animal date back to Latreille in 1817, where mites infesting a wombat held at the Museum National d'Histoire Naturelle in Paris were identified as morphologically identical to *Sarcoptes scabiei* found on a human; however, it is possible that mange was contracted during transportation (referenced in Skerratt, 1998).

Wombats severely infested with mange are generally found to be emaciated, having severe alopecia, and a thick dry crust com-posed of keratin (hyperkeratotic and/or parakeratotic) (Neste & Lachapelle, 1981; Skerratt, 2001; Van Neste & Staquet, 1986), containing bacteria, mites and neutrophilic and associated debris (Presidente, 1982; Skerratt, Martin, & Handasyde, 1998; Sweatman, 1971). Due to subcutaneous tunnelling in the skin, severe irritation occurs and the wombat experiences pruritus, suggestive of hyper-sensitivity (Arlian, 1989; Brugess, 1994; Skerratt, 2001). The infestation can be present on the whole body; however, the most affected areas are usually the head, neck, shoulders and limbs (Skerratt et al., 1998). Furthermore, haemorrhage, pyoderma and sometimes cutaneous myiasis have been seen to follow the damage of the underlying skin (Skerratt et al., 1998). Other mange-related impacts on the wombat include anaemia as a result of decreased haemoglobin, haematocrit and erythrocyte counts (Skerratt, 2001).

Ruykys et al. (2013) reported that mangy southern hairy-nosed wombats have elevated leucocyte counts, more specifically higher neutrophil and lymphocyte values. Biochemical values of mange-affected southern hairy-nosed wombats indicated that they had lower bicarbonate values when compared to unaffected wombats. The depressed bicarbonate value suggests that, on average, diseased wombats had lower metabolic rates than healthy wombats, perhaps due to reduced metabolic efficiency or energy intake (Ruykys et al., 2013).

Skerratt, Skerratt, Martin, and Handasyde (2004), Borchard, Eldridge, and Wright (2012), Simpson, Johnson, and Carver (2016) and personal observations have noted sarcoptic mange has both behavioural and physiological effects on wombats. Most commonly, and unusually for a nocturnal species, affected wombats are observed outside the burrow usually foraging during the day (Skerratt, Middleton, & Beveridge, 1999). Wombats with mange therefore lack nutrition, weigh less and have comparatively less subcutaneous fat compared to healthy wombats (Skerratt et al.,1999). Further, Simpson et al. (2016) have recently utilized thermal images taken with a high-resolution camera to investigate mange infested wombats and found that they tended to lose more body heat compared to healthy wombats. The study also confirmed severely affected wombats were more likely to have increased diurnal activity com-pared to healthy wombats, which might be because they spent increased time foraging. Simpson et al. (2016) suggested increased foraging was required to counteract the increased immunological and thermal costs associated with sarcoptic mange.

It is currently unknown why wombats are so severely impacted by sarcoptic mange; however, several theories have been suggested. Mange mites are thought to rely on close contact for transfer between hosts, and in humans, fomites from pillows and bed sheets can play an important role in mite transfer (Mellanby, 1985). Wombats frequent burrows that can be utilized by a range of different species (Old, Hunter, & Wolfenden, 2017) including the European red foxes and dogs. Skerratt et al. (2004) have also confirmed that wombats severely affected with parakeratotic sarcoptic mange can transmit Sarcoptes scabiei to other wombats.

European red foxes were introduced into Australia in 1850 and are well-recognized hosts of *Sarcoptes scabiei* (McCarthy, 1960). As mites can survive in low temperatures and high relative humidity for extended periods of time, and potentially up to 3 weeks (Arlian, 1989), and it has been documented that canids periodically enter wombat burrows (Triggs, 1998), it is possible that the route for transmission between both canids and wombats occurs via burrows (Skerratt et al., 1998). Furthermore, domestic dogs have been shown to contract mange after predating upon mangy wombats (Gray, 1937). Some suggestions have been made that canids may be necessary for disease persistence in marsupials (Roger, Laffan, & Ramp, 2011; Skerratt, 2005), but this requires further investigation. Also, persistent disease is observed in bare-nosed wombats on islands, where foxes are absent (Martin et al., 1998). Thus, evidence suggests mange can persist in wombats, and possibly other wildlife, with or without the involvement of canids. Nevertheless, to fully under-stand the reasons of susceptibility of wombats to sarcoptic mange, further studies are required.

One health is an emerging field of research that utilizes networking between health professionals and cutting-edge research tools (Thompson, 2013). Evaluating wombat health holistically should include assessment of stress levels and investigations into their immune system. The stress endocrine system, hypothalamo-pituitary adrenal (HPA) axis, is responsible for the regulation of physiological stress responses towards environmental stressors and can lead to changes in metabolism, immune-competence and behaviour in wild-life (see review by Narayan, 2017). As glucocorticoids can be measured non-invasively using faecal based assays (Fanson et al., 2017), monitoring stress levels using this method will provide valuable insights into the role of stress in sarcoptic mange susceptibility.

Whilst there have been papers describing sarcoptic mange infestations in wombats (such as the work of Skerratt referenced earlier in this paper), the literature is completely lacking any other information about the wombat immune system. Given the levels of major histocompatibility complex (MHC), gene diversity has been correlated to an animal's ability to combat disease and parasites in other species, including marsupials (Hermsen, Young, & Old, 2016; Lau, Jobbins, Belov, & Higgins, 2013; Smith, Belov, & Hughes, 2010); it would be pertinent that this is where studies begin into the wombat immune system.

2.5 | CURRENT TREATMENTS OF MANGE IN WOMBATS

It is clear that bare-nosed wombats have been impacted by sarcoptic mange mites, and as sarcoptic mange has been classified as an emerging infectious disease (Tompkins et al., 2015), it has raised concern amongst wildlife carers and zoologists (Martin et al., 1998; Tompkins et al., 2015). Community groups (Gilbert, 2017) and concerned citizens (Beavis, 2016) have therefore started treating mange-affected wombats with acaricides.

Fumigating burrows to kill the mites is not practical, thus treating wombats with an acaricide is the most viable alternative (Skerratt, 2001). There are several acaricides that are commercially available for the treatment of mange—ivermectin, moxidectin and selamectin. Treatment with ivermectin in domestic and wild animals is effective (Pence & Ueckermann, 2002). The drug usually affects the nerve impulses of the parasite thereby resulting in paralysis and ultimately death (Victoria & Trujillo, 2001).

In wombats, the most effective method to reduce the intensity of infection and eliminate mites is to treat affected wombat in captivity with two acaricides, one systemic and one topical (Skerratt, 2001). In captivity, it has been observed that to kill the larva of the parasite yet to emerge from the drug-resistant ova, a treatment regime of two to three medications 10 days apart is favoured (Cur-tis, 2004). Skerratt et al. (2004) found that one injection (0.4 mg/kg of ivermectin) in captive wombats was enough to remove mild mange; however, severely affected wombats required more treat-ments with ivermectin.

Although the sample size was small, efficacy of ivermectin was tested in a study of southern hairy-nosed wombats by Ruykys et al. (2013). The study revealed that a single dose of ivermectin can cure severely diseased wombats in captivity and mildly infected wombats in the wild (Ruykys et al., 2013). However, difficulties in both recapturing and holding wild individuals in captivity usually make such treatment courses impractical. Further, it is likely once treated, and they are released back into the same mite-infested environment that they will become infected again.

Methods designed to deliver acaricides non-invasively such as the “burrow-flap” method utilized by the Wombat Protection Society of Australia, Mange Management Incorporated and the Wombat Awareness Organization, and the “pole and scoop” technique has been developed to treat mange-affected wombats in the field. Using the “burrow flap” method, a trial treatment on a wild population of bare-nosed wombats infested with Sarcoptes scabiei was conducted in the Wolgan Valley Conservation Reserve, Newnes, NSW (Wolfen-den & Old, 2012). Based on McIlroy (1973) and observations using infrared cameras, 40 active burrows (20 each at two treatment sites where high numbers of wombats with mange were identified through spotlighting) were maintained over a 3-month trial period. Treatment stations (“burrow flaps”) mounted at burrow entrances were loaded with topical Cydectin© (Virbac Australia, Milperra, NSW), a commercially available antiparasitic “pour on” treatment typically used in the Australian agricultural sector as a low toxic therapy for cattle of all ages. When the treatment station was triggered, it was capable of delivering 4 ml of Cydectin© and was based on the recommended weekly dose level of 0.5 mg of Cydectin© per kilo-gram and an average wombat body weight of 25 kg. Over a 14-week period between late July and October 2011, the treatment
stations (10 stations per area) were monitored closely and recharged with 4 ml of Cydectin© every week on an alternating cycle. The treatment stations were positioned in groups of 5–10 to maximize the chance of treating a mange-affected animal at least once during a 1-week recharge period. To habituate the wombats with these stations, the reservoir of the flap was left empty for the first 7 days.

To determine whether there were any changes in the levels of mange in the population as a result of installation of the treatment stations, spotlighting surveys were carried out on two, eight, thirteen weeks post-treatment along set transects. Over the study period, a total of two hundred and eighty 4 ml doses were delivered across the 40 active burrows (Wolfenden & Old, 2012). However, despite installations of the treatment stations, the follow-up spotlighting surveys revealed no change in the mange level in the affected wombats, and the effect of Cydectin© as a successful treatment of sarcoptic mange for wild wombats using the treatment stations was not substantiated based on the results obtained in the study (Wolfenden & Old, 2012).

Wolfenden and Old (2012) found several limitations when using the treatment stations. Some wombats required up to 5 days getting accustomed to the presence of the treatment station at the entrance of their burrows and was observed during the pre-treatment surveys using infrared cameras and performing burrow inspections. The wombats often damaged the station resulting in replacement of 20% of the initial flaps that were installed and some stations had to be replaced within 3 weeks (Wolfenden & Old, 2012).

Bare-nosed wombats are known to share burrows and utilize multiple burrows at any one time (Skerratt et al.,1998). Therefore, despite the treatment stations being an effective and simple method of delivering an acaricide, only a few, if any, mange-affected wombats were treated in the study by Wolfenden and Old (2012). Further, the ratio of treatment stations to the number of active burrows and wombats was very small, and due to the very large population of wombats in the study area, and without intensive observations, it was difficult to determine which wombat resides in which burrows. Therefore, in the absence of such labour-intensive observations, there would be a high chance of treating a non-mange infested wombat. For successful treatment using the treatment stations in an area with large numbers of wombats, daily monitoring of the stations is needed and more thorough treatment procedures should be followed, in addition to locating the mange-affected wombats daily. Failure to do this increases the possibility of partial treatment of the wombats or mites developing a resistance to the parasite treatments (Bates, 1998). *Sarcoptes scabiei* has been reported to become increasingly drug-resistant in countries where acaricides had been previously been used to cure the disease (Bradberry, Cage, Proud-foot, & Vale, 2005; Currie, Harumal, McKinnon, & Walton, 2004; Sanderson et al., 2007). Some acaricides (e.g., ivermectin) have also been reported to have side effects, with Skerratt (2001) reporting some neurological disorders in wombats.

The possibility of mite resistance against acaricides has made it necessary to search for alternate therapies by testing and identifying novel chemotherapeutics (Heukelbach et al., 2004; Lawrence et al., 2005). Researchers have started investigating the role of glutathione transferases and other proteins in conferring acaricide resistance to Sarcoptes scabiei (Holt et al., 2003; Mounsey, Holt, McCarthy, Currie, Walton, 2008; Mounsey et al., 2010; Pasay et al., 2008). Another avenue may be the development of a vaccine. Skerratt (2001) suggested that sarcoptic mange infected wombats undergo an acquired immune response, and consequently, the feasibility of vaccinating wombats with an antigen from *Sarcoptes scabiei* could be investigated. Immunological cross-reactivity studies have shown that mites from different hosts produce identical proteins that are both variety specific and shared by different mite subtypes (Arlian, Morgan, & Arends, 1996). However, one of the main limitations to the development of a vaccine against mange is knowledge and selection of a protective antigen or antigens (Liu, Walton, & Mounsey, 2014). Knowledge of recombinant apolipoprotein (Harumal et al., 2003), glutathione S transferases (Pettersson, Ljunggren, Morrison, & Mattsson, 2005), serine proteases (Beckham et al., 2009; Holt et al., 2003), cysteine proteases (Walton et al., 2010) and serine protease inhibitors (Mika et al., 2012) of *Sarcoptes scabiei* may provide a promising candidate for the vaccine.

A study in goats (*Capra hircus*) has shown that vaccination with soluble proteins of *Sarcoptes scabiei* induced high levels of scabies-specific IgG in the serum of all animals but did not induce a specific IgE response. The immunocompetent goats were therefore not protected against mite challenge, but due to the development of a strong serum IgE and IgG response to the scabies antigen were resistant to reinfestation (Tarigan & Huntley, 2005). Similar results have been established in sheep (*Ovis aries*), where the animals were challenged with *Sarcoptes scabiei var. ovis*. The compromised sheep developed both IgG and IgE responses to mites. Following a secondary infestation, sheep developed a smaller area of mange lesion than that seen during primary challenge, and live Sarcoptes scabiei mites were not detected in skin samples (Rodriguez-Cadenas, Carbajal-Gonzalez, Fregeneda-Grandes, Aller-Gancedo, & Rojo-Vazquez, 2010).

Prior to some of this research progressing, there is a need to gain a better understanding of sarcoptic mange including its distribution in the wombat population. Martin et al. (1998), the only study that has investigated sarcoptic mange distribution, was conducted nearly two decades ago. Martin et al. (1998) determined sarcoptic mange occurred throughout the entire range of bare-nosed wombats, but the disease was relatively less prevalent in southern hairy-nosed wombats and absent in critically endangered northern hairy-nosed wombats. No nationwide survey has since been conducted to assess the changes in sarcoptic mange distribution and abundance in the wombat populations, yet a detailed map of sarcoptic mange prevalence is essential to gain a wider understanding of the disease and to manage the problem. Recently, a new mobile application called WomSAT (www.womsat.org.au) has been developed for this purpose. The application uses the in-built GPS system of any smartphone to report wombat sightings and uploads the information onto a national map. The data are collected in real-time and are not static like that collected using traditional survey techniques. The use of this new technology will identify factors that impact sarcoptic mange prevalence and distribution, and aid in our understanding of the disease process.

Overall, there is a lack of scientific funding for wombats compared to other more well-known Australian marsupials such as the koala (Tisdell & Nantha, 2007). For now, the critically endangered northern hairy-nosed wombat has not yet succumbed to sarcoptic mange (Skerratt et al., 1998); however, if the populations were to become infested, it could have dire consequences for the species. Research into the immunology of wombats and sarcoptic mange is critical, especially given that wombat populations are already being negatively impacted by habitat loss and other human-induced threats.

2.6 | CONCLUSIONS

Sarcoptic mange is the major cause of mortality in bare-nosed and southern hairy-nosed wombats. In the absence of a more thorough treatment, more wombats will succumb to sarcoptic mange. Monitoring wombat populations and gaining more knowledge of wombat immunology and sarcoptic mange will aid our understanding of this disease in wombats and the development of future management strategies for the species.

CONFLICTOFINTEREST

The authors have no conflicts of interest.

2.7 | REFERENCES

Alasaad, S., Permunian, R., Gakuya, F., Mutinda, M., Soriguer, R. C., & Rossi, L. (2012). Sarcoptic-mange detector dogs used to identify infected animals during outbreaks in wildlife. Veterinary Research 8, 110–116.

Alasaad, S., Soglia, D., Sarasa, M., Soriguer, R. C., Perez, J. M., Granados, J. E., & Rossi, L. (2008). Skin-scale genetic structure of *Sarcoptes scabiei* populations from individual hosts: Empirical evidence from Iberian ibex-derived mites. Parasitology Research, 104, 101–105. https://doi.org/10.1007/s00436-008-1165-3

Alasaad, S., Walton, S., Rossi, L., Bornstein, S., Abu-Madi, M., Soriguer, R. C., & Ugbomoiko, U. S. (2011). Sarcoptes-world molecular network (Sarcoptes-WMN): Integrating research on scabies. International Journal of Infectious Diseases, 15, e294–e297. https://doi.org/10.1016/j.ijid.2011.01.012

Alexander, J. O. D. (1984). Scabies. In Arthropods and human skin. London: Springer-Verlag. https://doi.org/10.1007/978-1-4471-1356-0 Amer, S., El Wahab, T. A., Metwaly, A. E. N., Ye, J., Roellig, D., Feng, Y.&,Xiao, L. (2014). Preliminary molecular characterizations of *Sarcoptes scabiei* (Acari: Sarcoptidae) from farm animals in Egypt. PLoS ONE, 9, e94705. https://doi.org/10.1371/journal.pone.0094705

Arlian, L. G. (1989). Biology, host relations, and epidemiology of *Sarcoptes scabiei.* Annual Review of Entomology, 34, 139–159. https://doi.org/ 10.1146/annurev.en.34.010189.001035

Arlian, L. G., Morgan, M. S., & Arends, J. J. (1996). Immunologic cross-reactivity among various strains of *Sarcoptes scabiei*. Journal of Parasitology, 82, 66–72. https://doi.org/10.2307/3284117

Arlian, L., Vyszenski-Moher, D., & Pole, M. (1989). Survival of adults and developmental stages of *Sarcoptes scabiei var. canis* when off the host. Experimental and Applied Acarology, 6, 181–187. https://doi.org/10.1007/BF01193978

Arundel, J., Barker, I., & Beveridge, I. (1977). Diseases of marsupials. In B. Stonehouse & D. Gilmore (Eds.), The biology of marsupials. London: Academic Press.

Barbet, J. L. (2014). Ectoparasites of Horses. In D. C. Sellon & M. T. Long (Eds.), Equine Infectious Diseases, 2nd ed. St Louis, MO: W.B. Saunders.

Barker, I. (1974). *Sarcoptes scabiei* infestation of a koala (*Phascolarctos cinereus*), with probable human involvement. Australian Veterinary Journal, 50, 528. https://doi.org/10.1111/j.1751-0813.1974.tb14068.x

Bates, P. S. (1998). Acaricide resistance in sheep scab mites. Proceeding of the Sheep Veterinary Society, 21, 117–122.

Beavis, L. (2016) Wombat mange prompts Tasmanian residents to take lead on tackling lethal disease. Retrieved from http://www.abc.net.au/news/2016-12-18/kelso-residents-take-lead-on-tackling-wombat-mange/8130184

Beckham, S. A., Boyd, S. E., Reynolds, S., Willis, C., Johnstone, M., Mika, A., Kemp, D. J. (2009). Characterization of a serine protease homologous to house dust mite group 3 allergens from the scabies mite Sarcoptes scabiei. Journal of Biological Chemistry, 284, 34413– 34422. https://doi.org/10.1074/jbc.M109.061911

Berrilli, F., D'Amelio, S., & Rossi, L. (2002). Ribosomal and mitochondrial DNA sequence variation in Sarcoptes mites from different hosts and geographical regions. Parasitology Research, 88, 772–777.

Borchard, P., Eldridge, D. J., & Wright, I. A. (2012). Sarcoptes mange *(Sarcoptes scabiei*) increases diurnal activity of bare-nosed wombats (Vombatus ursinus) in an agricultural riparian environment. Mammalian Biol-Zeitschrift fur Saugetierkunde, 77, 244–248. https://doi.org/10.1016/j.mambio.2012.04.004

Bornstein, S., Morner, T., & Samuel, W. M. (2001). *Sarcoptes scabiei* and sarcoptic mange. In W. M. Samuel, M. J. Pybus, & A. A. Kocan (Eds.), Parasitic Diseases of wild mammals, 2nd ed. (pp. 107–119). Ames: Iowa State University Press. https://doi.org/10.1002/9780470377000

Bornstein, S., Zakrisson, G., & Thebo, P. (1995). Clinical picture and antibody response to experimental *Sarcoptes scabiei var. vulpes* infection in red foxes (*Vulpes vulpes*). Acta Veterinaria Scandinavica, 36, 509– 519.

Bradberry, S. M., Cage, S. A., Proudfoot, A. T., & Vale, J. A. (2005). Poisoning due to pyrethroids. Toxicological Reviews, 24, 93–106. https://doi.org/10.2165/00139709-200524020-00003

Bretz, M. (2012). The Role of Bandicoot Foraging Pits in Ecosystem Processes. Honours thesis, Murdoch University, Perth, Australia.

Brown, A. (2004). A review of camel diseases in Central Australia. Retrieved from https://dpir.nt.gov.au/__data/assets/pdf_file/0014/233222/tb314.pdf

Brugess, I. (1994). *Sarcoptes scabiei* and scabies. Advances in Parasitology, 33, 235–292. https://doi.org/10.1016/S0065-308X(08)60414-5

Castro, I., de la Fuente, A., Fandos, P., Cano-Manuel, F., Granados, J.-E., & Soriguer, R. C. (2016). *Sarcoptes scabiei* infesting Iberian ibex (Capra pyrenaica). International Journal of Acarology, 42, 7–11. https://doi.org/10.1080/01647954.2015.1109710

Currie, B. J., Harumal, P., McKinnon, M., & Walton, S. F. (2004). First documentation of in vivo and in vitro ivermectin resistance in Sarcoptes scabiei. Clinical and Infectious Diseases, 39, e8–e12. https://doi.org/10.1086/421776

Curtis, C. F. (2004). Current trends in the treatment of Sarcoptes, Cheyletiella and Otodectes mite infestations in dogs and cats. Veterinary Dermatology, 15, 108–114. https://doi.org/10.1111/vde.2004.15.issue-2

Damriyasa, I., Failing, K., Volmer, R., Zahner, H., & Bauer, C. (2004). Prevalence, risk factors and economic importance of infestations with *Sarcoptes scabiei* and *Haematopinus suis* in sows of pig breeding farms in Hesse, Germany. Medical and Veterinary Entomology, 18, 361–367. https://doi.org/10.1111/mve.2004.18.issue-4

Danell, K., & Hornfeldt, B. (1987). Numerical responses by populations of red fox and mountain hare during an outbreak of sarcoptic mange. Oecologia, 73, 533–536. https://doi.org/10.1007/BF00379412

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2000). Emerging infectious diseases of wildlife-threats to biodiversity and human health. Science, 287, 443. https://doi.org/10.1126/science.287.5452.443

Davies, P. R. (1995). Sarcoptic mange and production performance of swine: A review of the literature and studies of associations between mite infestation, growth rate and measures of mange severity in growing pigs. Veterinary Parasitology, 60, 249–264. https://doi.org/ 10.1016/0304-4017(95)00795-3

Davies, R. R., Moore, M. J., & Pointon, A. M. (1991). Seasonality of sarcoptic mange in pigs in South Australia. Australian Veterinary Journal, 68, 390–392. https://doi.org/10.1111/j.1751-0813.1991.tb03105.x

Dickman, C. R. (2006). Positive effects of rodents on biota in arid Australian systems. Canberra, ACT: Centre for International Agricultural Research.

Donahoe, S. L., Slapeta, J., Knowles, G., Obendorf, D., Peck, S., & Phalen, D. N. (2015). Clinical and pathological features of toxoplasmosis in free-ranging common wombats (*Vombatus ursinus*) with multilocus genotyping of Toxoplasma gondii type II-like strains. Parasitology Inter-national, 64, 148–153. https://doi.org/10.1016/j.parint.2014.11.008

Eshar, D. (2010). Prevalence of sarcoptic mange in pet rabbits (*Oryctolagus cuniculus*) in Israel. Israel Journal of Veterinary Medicine, 65, 140.

Fain, A. (1968). Etude de la variabilite de *Sarcoptes scabiei* avec une revision des Sarcoptidae. Acta Zoology and Pathology Antverp, 47, 1–196.

Fain, A. (1978). Epidemiological problems of scabies. International Journal of Dermatology, 17, 20–30. https://doi.org/10.1111/ijd.1978.17.issue-1

Fanson, K., Best, E. C., Bunce, A., Fanson, B. G., Hogan, L. A., Keeley, T. Bashaw, M. (2017). One size does not fit all: Monitoring faecal glucocorticoid metabolites in marsupials. General and Comparative Endocrinology, 244, 146–156. https://doi.org/10.1016/j.ygcen.2015.10.011

Fleming, P. A., Anderson, H., Prendergast, A. S., Bretz, M. R., Valentine, L. E., & Hardy, G. E. (2014). Is the loss of Australian digging mammals contributing to a deterioration in ecosystem function? Mammal Review, 44, 94–108. https://doi.org/10.1111/mam.2014.44.issue-2

Fleming, P., Corbett, L., Harden, R., & Thompson, P. (2001). Managing the Impact of Dingoes and Other Wild Dogs. Canberra, ACT: Australian Government Publishing.

Fraser, T. A., Charleston, M., Martin, A., Polkinghorne, A., & Carver, S. (2016). The emergence of sarcoptic mange in Australian wildlife: An unresolved debate. Parasites & Vectors, 9, 316. https://doi.org/10.1186/s13071-016-1578-2

Gilbert, T. O. E. (2017). Wombat Mange: Call for volunteers to help treat animals 'itching to death' in Canberra. Retrieved from http://www.abc.net.au/news/2017-02-15/call-for-canberra-volunteers-to-treat-wombat-mange/8273562.

Gray, D. (1937). Sarcoptic mange affecting wild fauna in New South Wales. Australian Veterinary Journal, 13, 154–155. https://doi.org/10.1111/j.1751-0813. 1937.tb04110.x

Gu, X.-B., & Yang, G.-Y. (2008). A study on the genetic relationship of mites in the genus Sarcoptes (Acari: Sarcoptidae) in China. International Journal of Acarology, 34, 183–190. https://doi.org/10.1080/01647950808683722

Gutierrez, J. F., de Vigo, J. M., Castella, J., Munoz, E., & Ferrer, D. (1996). Prevalence of sarcoptic mange in fattening pigs sacrificed in a slaughterhouse of northeastern Spain. Veterinary Parasitology, 61, 145–149. https://doi.org/10.1016/0304-4017(95)00814-4

Harumal, P., Morgan, M., Walton, S. F., Holt, D. C., Rode, J., Arlian, L. G., Kemp, D. J. (2003). Identification of a homologue of a house dust mite allergen in a cDNA library from *Sarcoptes scabiei var. hominis* and evaluation of its vaccine potential in a rabbit/*S. scabiei var. canis* model. American Journal of Tropical Medicine & Hygiene, 68, 54–60.

Hermsen, E., Young, L., & Old, J. (2016). Major Histocompatibility Complex class II in the red-tailed phascogale (*Phascogale calura*). Australian Mammalogy, 39, 28–32.

Heukelbach, J., Winter, B., Wilcke, T., Muehlen, M., Albrecht, S., Sales de Oliveira, F. A., Feldmeier, H. (2004). Selective mass treatment with

ivermectin to control intestinal helminthiases and parasitic skin diseases in a severely affected population. Bulletin World Health Organisation, 82, 563–571.

Holt, D. C., Wilson, P., Slade, R., Currie, B. J., Walton, S. F., Fischer, K., Kemp, D. J. (2003). Mechanisms for a novel immune evasion strategy in the scabies mite *Sarcoptes scabiei*: A multigene family of inactivated serine proteases. Journal of Investigative Dermatology, 121, 1419–1424.

Johnson, C., & Gordon, G. (1995). Northern hairy-nosed wombat. In The Australian Museum Complete (Ed.), Book of Australian Mammals. Sydney, NSW: Reed Books.

Jones, C. G., Lawton, J. H., & Shachak, M. (1997). Positive and negative effects of organisms as physical engineers. Ecology, 78, 1946–1957. https://doi.org/10.1890/0012-9658(1997)078[1946:PANEOO]2.0.CO;2

Kambarage, D., Msolla, P., & Falmer-Hansen, J. (1990). Epidemiological studies of sarcoptic mange in Tanzanian pig herds. Tropical Animal Health Production, 22, 226–230. https://doi.org/10.1007/BF02240404

Kinlaw, A. (1999). A review of burrowing by semi-fossorial vertebrates in arid environments. Journal of Arid Environments, 41, 127–145. https://doi.org/10.1006/jare.1998.0476

Kotb, S., & Abdel-Rady, A. (2015). Sarcoptic mange of camel in upper Egypt: Prevalence, risk assessment, and control measures. Journal of Advanced Veterinary Animal Research, 2, 410–417. https://doi.org/10.5455/javar.

Lau, Q., Jobbins, S. E., Belov, K., & Higgins, D. (2013). Characterisation of four major histocompatibility complex class II genes of the koala (*Phascolarctos cinereus*). Immunogenetics, 65, 37–46. https://doi.org/10.1007/s00251-012-0658-5

Lawrence, G., Leafasia, J., Sheridan, J., Hills, S., Wate, J., Wate, C., Purdie, D. (2005). Control of scabies, skin sores and haematuria in children in the Solomon Islands: Another role for ivermectin. Bulletin of the World Health Organisation, 83, 34–42.

Lindstrom, E. R., Andren, H., Angelstam, P., Cederlund, G., Hornfeldt, B., Jeaderberg, L., Swenson, J. E. (1994). Disease reveals the predator: Sarcoptic mange, red fox predation, and prey populations. Ecology, 75, 1042–1049. https://doi.org/10.2307/1939428

Little, S. E., Davidson, W. R., Howerth, E. W., Rakich, P. M., & Nettles, V. F. (1998). Diseases diagnosed in red foxes from the southeastern United States. Journal of Wildlife Diseases, 34, 620–624. https://doi.org/10.7589/0090-3558-34.3.620

Liu, X., Walton, S., & Mounsey, K. (2014). Vaccine against scabies: Necessity and possibility. Parasitology, 141, 725–732. https://doi.org/10.1017/S0031182013002047

Marks, C. A. (1998). Review of the humaneness of destruction techniques used on the common wombat *Vombatus ursinus* in Victoria. In R. T. Wells, P. A. Pridmore (Eds.), Wombats. Chipping Norton: Surrey Beatty and Sons.

Martin, R., Handasyde, K., & Skerratt, L. (1998). Current distribution of sarcoptic mange in wombats. Australia Veterinary Journal, 76, 411–414https://doi.org/10.1111/j.1751-0813.1998.tb12391.x

McCarthy, P. (1960). The presence of sarcoptic mange in the wild fox (Vulpes vulpes) in Central Queensland. Australia Veterinary Journal, 36, 359–360. https://doi.org/10.1111/j.1751-0813.1960.tb08669.x

McIlroy, J. C. (1973). Aspects of the Ecology of the Common Wombat, *Vombatus ursinus* (Shaw, 1800). PhD thesis, Australian National University.

McIlroy, J. (1995). Common wombat. The mammals of Australia. Chatswood, NSW: Reed Book.

McLelland, D., & Youl, J. (2005). Sarcoptic mange in agile wallabies (*Macropus agilis*) in the Northern Territory. Australian Veterinary Journal, 83, 744–745. https://doi.org/10.1111/j.1751-0813.2005.tb11585.x

Mellanby, K. (1985). Epidemiology of scabies. In M. Orkin & H. I. Maibach (Eds.), Cutaneous infestations and insect bites. New York, USA: Basel: Marcel Dekker Incorporated.

Mika, A., Reynolds, S. L., Mohlin, F. C., Willis, C., Swe, P. M., Pickering, D. A., Blom, A. M. (2012). Novel scabies mite serpins inhibit the three pathways of the human complement system. PLoS ONE, 7, e40489. https://doi.org/10.1371/journal.pone.0040489

Mounsey, K. E., Holt, D. C., McCarthy, J., Currie, B. J., & Walton, S. F. (2008). Scabies: Molecular perspectives and therapeutic implications in the face of emerging drug resistance. Future Microbiology, 3, 57– 66. https://doi.org/10.2217/17460913.3.1.57

Mounsey, K. E., Murray, H. C., Bielefeldt-Ohmann, H., Pasay, C., Holt, D. C., Currie, B. J., McCarthy, J. S. (2015). Prospective study in a por-cine model of *Sarcoptes scabiei* indicates the association of Th2 and Th17 pathways with the clinical severity of scabies. PLoS Neglected Tropical Diseases, 9, e0003498.

Mounsey, K. E., Pasay, C. J., Arlian, L. G., Morgan, M. S., Holt, D. C., Currie, B. J., McCarthy, J. S. (2010). Increased transcription of Glutathione S-transferases in acaricide exposed scabies mites. Parasites & Vectors, 3, 43. https://doi.org/10.1186/1756-3305-3-43

Munang'andu, H. M., Siamudaala, V. M., Matandiko, W., Munyeme, M., Chembensofu, M., & Mwase, E. (2010). Sarcoptes mite epidemiology and treatment in African buffalo (*Syncerus caffer*) calves captured for translocation from the Kafue game management area to game ranches. BMC Veterinary Research, 6, 29. https://doi.org/10.1186/1746-6148-6-29

Narayan, E. J. (2017). Evaluation of physiological stress in Australian wildlife: Embracing pioneering and current knowledge as a guide to future research directions. General and Comparative Endocrinology, 244, 30–39. https://doi.org/10.1016/j.ygcen.2015.12.008

Neste, D., & Lachapelle, J. (1981). Host parasite relationships in hyperkeratotic (Norwegian) scabies: Pathological and immunological findings. British Journal of Dermatology, 105, 667–678. https://doi.org/10.1111/ bjd.1981. 105.issue-6

Obendorf, D. L. (1983). Causes of mortality and morbidity of wild koalas, *Phascolarctos cinereus* (Goldfuss), in Victoria, Australia. Journal of Wildlife Diseases, 19, 123–131. https://doi.org/10.7589/0090-3558-19.2.123

Old, J. M., Hunter, N. E., & Wolfenden, J. (2017). Who utilises bare-nosed wombat burrows?. Australian Zoologist: In Press.

Pasay, C., Arlian, L., Morgan, M., Vyszenski-Moher, D., Rose, A., Holt, D., McCarthy, J. (2008). High resolution melt analysis for the detection of a mutation associated with permethrin resistance in a population of scabies mites. Medical and Veterinary Entomology, 22, 82–88. https://doi.org/10.1111/j.1365-2915.2008.00716.x

Pence, D. B., & Ueckermann, E. (2002). Sarcoptic mange in wildlife and Technical Review of the Office International des Epizooties (Paris), 21, 385–398.

Pence, D. B., & Windberg, L. A. (1994). Impact of a sarcoptic mange epizootic on a coyote population. Journal of Wildlife Management, 58, 624–633. https://doi.org/10.2307/3809675

Pettersson, E. U., Ljunggren, E. L., Morrison, D. A., & Mattsson, J. G. (2005). Functional analysis and localisation of a delta-class glutathione S-transferase from *Sarcoptes scabiei*. International Journal of Parasitology, 35, 39–48. https://doi.org/10.1016/j.ijpara.2004.09.006

Presidente, P. (1982). Common wombat *Vombatus ursinus*: Maintenance in captivity, blood values, infectious and parasitic diseases. In D. D. Evans (Ed.), The management of Australian mammals in captivity. Melbourne, VIC: Zoological Parks Board of Victoria

Railliet, A. (1895). Traite de zoologie medicale agricole. Paris: Asselin and Houzeaux. https://doi.org/10.5962/bhl.title.34139

Rodriguez-Cadenas, F., Carbajal-Gonzalez, M., Fregeneda-Grandes, J., Aller-Gancedo, J., & Rojo-Vazquez, F. A. (2010). Clinical evaluation and antibody responses in sheep after primary and secondary experimental challenges with the mange mite *Sarcoptes scabiei var. ovis*. Veterinary Immunology and Immunopathology, 133, 109–116. https://doi.org/10.1016/j.vetimm.2009.07.004

Roger, E., Bino, G., & Ramp D. (2012). Linking habitat suitability and road mortalities across geographic ranges. Landscape Ecology, 27(8), 1167–1181. https://doi.org/10.1007/s10980-012-9769-5

Roger, E., Laffan, S. W., & Ramp, D. (2011). Road impacts a tipping point for wildlife populations in threatened landscapes. Population Ecology, 53, 215–227. https://doi.org/10.1007/s10144-010-0209-6

Ruykys, L., Breed, B., Schultz, D., & Taggart, D. (2013). Effects and treatment of sarcoptic mange in southern hairy-nosed wombats (*Lasiorhinus latifrons*). Journal of Wildlife Diseases, 49, 312–320. https://doi. org/10.7589/2012-10-256

Sanderson, H., Laird, B., Pope, L., Brain, R., Wilson, C., Johnson, D., Solomon, K. (2007). Assessment of the environmental fate and effects of

ivermectin in aquatic mesocosms. Aquatic Toxicology, 85, 229–240. https://doi.org/10.1016/j.aquatox.2007.08.011

Saunders, G., Coman, B., Kinnear, J., & Braysher, M. (1995). Managing vertebrate pests: Foxes. Canberra: Australian Government Publishing Service.

Simpson, K., Johnson, C. N., & Carver, S. (2016). Sarcoptes scabiei: The mange mite with mighty effects on the common wombat (*Vombatus ursinus*). PLoS ONE, 11, e0149749. https://doi.org/10.1371/journal.pone.0149749

Skerratt, L. F. (1998). Diseases and parasites of the common wombat *Vombatus ursinus* in the Healesville area of Victoria. In R. T. Wells & P. A. Pridmore (Eds.), Wombats. Chipping Norton, NSW: Surrey Beatty & Sons.

Skerratt, L. F. (2001). Sarcoptic mange in the common wombat, *Vombatus ursinus* (Shaw, 1800). PhD thesis, The University of Melbourne.

Skerratt, L. F. (2005). *Sarcoptes scabiei*: An important exotic pathogen of wombats. Microbiology Australia, 26, 79–81.

Skerratt, L., Campbell, N., Murrell, A., Walton, S., Kemp, D., & Barker, S. (2002). The mitochondrial 12S gene is a suitable marker of populations of *Sarcoptes scabiei* from wombats, dogs and humans in Australia. Parasitology Research, 88, 376–379. https://doi.org/10.1007/ s00436-001-0556-5

Skerratt, L., Martin, R., & Handasyde, K. (1998). Sarcoptic mange in wombats. Australian Veterinary Journal, 76, 408–410. https://doi.org/10.1111/j.1751-0813. 1998.tb12389.x

Skerratt, L. F., Middleton, D., & Beveridge, I. (1999). Distribution of life cycle stages of *Sarcoptes scabiei var wombati* and effects of severe mange on common wombats in Victoria. Journal of Wildlife Diseases, 35, 633–646. https://doi.org/10.7589/0090-3558-35.4.633

Skerratt, L. F., Skerratt, J. H., Martin, R., & Handasyde, K. (2004). The effects of sarcoptic mange on the behaviour of wild common wombats (Vombatus ursinus). Australian Journal of Zoology, 52, 331–339. https://doi.org/10.1071/ZO02062

Smales, L. (1998). Helminth parasites of wombats. In R. T. Wells & P. A. Pridmore (Eds.), Wombats. Chipping Norton, NSW: Surrey Beatty & Sons.

Smith, S., Belov, K., & Hughes, J. (2010). MHC screening for marsupial conservation: Extremely low levels of class II diversity indicate population vulnerability for an endangered Australian marsupial. Conservation Genetics, 1, 269–278. https://doi.org/10.1007/s10592-009-0029-4

Sweatman, G. K. (1971). Mites and pentasomes. In J. W. Davis & R. C. Anderson (Eds.), Parasitic diseases of wild mammals, Ames, Iowa: Iowa State Press.

Taggart, D., Martin, R., & Horsup, A. (2016a). *Lasiorhinus krefftii*. The IUCN Red List of Threatened Species 2016. Retrieved from https://doi.org/10.2305/iucn.uk.2016-2.rlts.t11343a21959050.en

Taggart, D., Martin, R., & Menkhorst, P. (2016b). *Vombatus ursinus*. The IUCN Red List of Threatened Species 2016. Retrieved from https://doi.org/10.2305/iucn.uk.2016-2.rlts.t40556a21958985.en

Tarigan, S., & Huntley, J. F. (2005). Failure to protect goats following vaccination with soluble proteins of *Sarcoptes scabiei*: Evidence for a role for IgE antibody in protection. Veterinary Parasitology, 133, 101–109. https://doi.org/10.1016/j.vetpar.2005.03.044

Temby, I. (1998). The law and wombats in Australia. In R. T. Wells & P. A. Pridmore (Eds.), Wombats. Chipping Norton, NSW: Surrey Beatty and Sons.

Thompson, R. C. A. (2013). Parasite zoonoses and wildlife: One health, spillover and human activity. International Journal of Parasitology, 43, 1079–1088. https://doi.org/10.1016/j.ijpara.2013.06.007

Tisdell, C., & Nantha, H. S. (2007). Comparison of funding and demand for the conservation of the charismatic koala with those for the critically endangered wombat *Lasiorhinus krefftii*. Biodiversity and Conservation, 16, 1261–1281. https://doi.org/10.1007/s10531-006-6735-z

Tompkins, D. M., Carver, S., Jones, M. E., Krkosek, M., & Skerratt, L. F. (2015). Emerging infectious diseases of wildlife: A critical perspective. Trends in Parasitology, 31, 149–159. https://doi.org/10.1016/j.pt.2015.01.007

Triggs, B. (1998). The wombat: Common wombats in Australia. Sydney, NSW: University of New South Wales Press.

Van Neste, D. J., & Staquet, M. J. (1986). Similar epidermal changes in hyperkeratotic scabies of humans and pigs. American Journal of Dermatopathology, 8, 267–273. https://doi.org/10.1097/00000372-198606000-00018

Victoria, J., & Trujillo, R. (2001). Topical ivermectin: A new successful treatment for scabies. Paediatric Dermatology, 18, 63–65. https://doi.org/10.1046/j.1525-1470.2001. 018001063.x

Walton, S. F., Choy, J. L., Bonson, A., Valle, A., McBroom, J., Taplin, D., Kemp, D. J. (1999). Genetically distinct dog-derived and human-derived *Sarcoptes scabiei* in scabies-endemic communities in northern Australia. American Journal of Tropical Medicine and Hygiene, 61, 542– 547. https://doi.org/10.4269/ajtmh.1999.61.542

Walton, S., Dougall, A., Pizzutto, S., Holt, D., Taplin, D., Arlian, L., Kemp, D. (2004). Genetic epidemiology of *Sarcoptes scabiei* (Acari: Sarcoptidae) in northern Australia. International Journal of Parasitology, 34, 839–849. https://doi.org/10.1016/j.ijpara.2004.04.002

Walton, S. F., Pizzutto, S., Slender, A., Viberg, L., Holt, D., Hales, B. J., O'Hehir, R. (2010). Increased allergic immune response to *Sarcoptes scabiei* antigens in crusted versus ordinary scabies. Clinical Vaccine and Immunology, 17, 1428–1438. https://doi.org/10.1128/CVI.00195-10

Woinarski, J., & Burbidge, A. A. (2016). *Lasiorhinus latifrons*. The IUCN Red List of Threatened Species 2016. Retrieved from https://doi.org/10.2305/iucn.uk.2016-1.rlts.t40555a21959203.en

Wolfenden, J., & Old, J. M. (2012). Taking action to improve wombat health on Emirates Estates Wolgan Conservation area. Report for Wolgan Valley Resort and Spa.

Womersley, H. (1953). On the Sarcoptid or mange-mites of the wombat. Records of the South Australian Museum, 11, 69–73.

Zahid, M., Maqbool, A., Anjum, S., Ashraf, K., Khan, M., & Ahmad, N. (2015). Prevalence of sarcoptic mange in camels in Punjab, Pakistan. Journal of Animal and Plant Science, 25, 1259–1263.

Zahler, M., Essig, A., Gothe, R., & Rinder, H. (1999). Molecular analyses suggest monospecificity of the genus Sarcoptes (Acari: Sarcoptidae). International Journal of Parasitology, 29, 759–766. https://doi.org/10.1016/S0020-7519(99)00034-X

Chapter 3: Testing the environmental decay of faecal cortisol metabolites in bare-nosed wombats (*Vombatus ursinus*)

3.1 Chapter Outline:

Chapter 3 evaluated the decay rate of faecal cortisol metabolites in voided faeces of bare-nosed wombats in autumn in NSW, Australia. This chapter validated two EIAs to accurately monitor stress physiology in bare-nosed wombats. The decay rate of faecal cortisol metabolites and the EIA validation results are important to future researchers who aim to study the stress physiology of bare-nosed wombats. This study was conducted with the approval Western Sydney University Animal Care and Ethics Committee (Protocol number: A12033) and WSU Biosafety and Radiation Safety Committee (Protocol number: B10524).

The following paper is jointly co-authored, where I am the primary and the corresponding author of this paper. I performed the faecal cortisol metabolites extraction from voided faecal samples of bare-nosed wombats and enzyme-immunoassays on the extracted faecal cortisol metabolites, analysed the data and drafted the manuscript. Associate Professor Julie Old is the second author of this paper and supervised the development of the manuscript drafts and provided editorial feedback on the manuscript drafts. Dr. Edward Narayan is the third author and helped in the development of experimental design and supervised the development of the study and the manuscript drafts.

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3.2. Introduction

Conservation endocrinology (Cockrem and Ishii, 1999) and conservation physiology (Wikelski and Cooke, 2006) have advanced techniques to assess the physiological responses of animals to anthropogenic induced environmental change. Non-invasive glucocorticoid monitoring is an important tool which has enabled researchers to monitor the adrenal activity of wild and captive animals that complements behavioural (Vaz et al., 2017), physiological (Rodrigues et al., 2015) and pathological studies (Kindermann et al., 2017). One such non-invasive technique that is being used widely in wildlife studies is faecal cortisol metabolite (FCM) enzyme immuno assay (EIA). Faecal sampling does not require capture and restraint of animals hence it is favoured over traditional methods of hormone profiling such as blood sampling (Dantzer et al., 2014, Millspaugh and Washburn, 2004, Sheriff et al., 2011). Like all biological agents, FCMs are also susceptible to bacterial decomposition and environmental factors such as cold, heat and precipitation (Terio et al., 2002, Washburn and Millspaugh, 2002). Multiple studies (see Table 1.1) have tried to address the issue of bacterial decomposition of wildlife faeces. In the wild, it is not always possible to obtain fresh faeces for experimentation and it is difficult to estimate the exact time since defecation (Evans et al., 2013). Therefore, validation studies are essential to understand the decay rate of hormone metabolites in voided faecal matter. An investigation of the decay rate of the hormone metabolites will help future researchers to recognise the optimal window for sample collection such that all samples collected within that time frame will provide accurate results representing baseline hormone levels of the animal concerned.

In this study, we examined the stability of FCMs in faecal samples collected from captive bare-nosed wombats (*Vombatus ursinus*). Bare-nosed wombats are distributed in New South Wales, Victoria, Australian Capital Territory, Flinders Island, the south-east of South Australia, south-east Queensland and Tasmania (Triggs, 2009). They are frequently exposed to diseases such

Table 3.1: Studies on Decay Rate of faecal Glucocorticoid Metabolites in Wildlife

Species	Scientific name	Assay used, Sample details	Objective	Stability of Faecal glucocorticoid metabolites	References
Japanese quail	*Coturnix japonica*	Cortisone EIA (antiserum 4-pregnene-17α,21-diol-3,11,20-trione-21-HS); n = 36; 36 M	Changes in faecal CM level with varying drying methods of samples	Mean CM concentrations increased over time (0<4<24=48 h p.d) in both oven dried and non-oven dried samples	Pellegrini et al. (2015)
Jaguars	*Panthera onca*	Cortisol EIA (antiserum R4866; C Munro, UCDavis USA); double-antibody ^{125}I-labelled corticosterone RIA (MP Biomedicals, Orangeburg, NY, USA); n = 10; 6 F and 4 M	Changes in FGM levels with varying environmental conditions (sun or shade) and seasons (dry and wet)	Mean FGM concentration remained stable for 5 d p.d during dry season while < 1 d p.d for wet season with cortisol EIA and for 5 d p.d in both dry and wet seasons with corticosterone RIA; shade and sun did not have any effect on FGM concentration	Mesa-Cruz et al. (2014)
mountain hare	*Lepus timidus*	11-oxo etiocholanolone EIA and one 5α-pregnane-3β, 11β, 21-triol-20-one EIA; n = 2; 1 M and 1 F	Changes in GCM levels with varying storage and temperature conditions (10ºC and 20ºC)	Mean GCM concentration level fluctuations were low within the storage period of 12,24,48 and 72 h p.d, both at 10ºC and 20ºC	Rehnus et al. (2009)
southern hairy-nosed wombat	*Lasiorhinus latifrons*	Progesterone, testosterone, corticosterone EIA (antibody CJM06; C Munro, UC Davis, USA); n = 12; 8 F and 4 M	Changes in progesterone, testosterone and corticosterone metabolite levels with time delay of sample freezing	Progesterone metabolite concentration showed progressive decrease with time, testosterone metabolite concentration showed significant increase after 6 h delay and corticosterone metabolite concentration increased significantly initially (0-6 h p.d), it remained at this level until 24 h and it dropped to its original level by 72 h	Descovich et al. (2012)
Asian elephant	*Elephas maximus*	Corticosterone EIA (antibody CJM06; C Munro, UC Davis, USA); n = 10; 8 F and 2 M	Changes in fGCM concentration with varying environmental (sun or shade) and storage conditions	Mean fGCM concentration level remained stable for up to 8 h p.d with subsequent increase by 1 d and then the level dropped below time 0 level by 2 d. Sun dried samples showed higher fGCM concentrations than those in shade.	Wong et al. (2016)
African elephant	*Loxodonta africana*	3α,11oxo-cortisol EIA; n = 2; 1 F and 1 M	Changes in fGCM concentration with varying environmental (sun or shade) and storage conditions	Mean fGCM concentration level remained stable for 20 h p.d with subsequent decrease with time; freeze-dried fGCM level >sun-dried fGCM level> shade-dried fGCM level.	Webber et al. (2018)

Tiger	*Panthera tigris*	Cortisol EIA (antiserum R4866; C Munro, UCDavis USA); n = 8; 2 F and 6 M	Changes in FCM concentration with time	Mean FCM concentration remained stable between 0-48 h p.d	Parnell et al. (2015)
Greater bilby	*Macrotis lagotis*	cortisol EIA (antiserum R4866; C Munro, UCDavis USA); n=7; 4 F and 3 M	Changes in FCM concentration with time	Mean FCM concentration remained fairly stable for 19 d p.d	Evanset al. (2013)
Western lowland gorilla	*Gorilla gorilla gorilla*	3a,11ßdihydroxy-cortisol metabolite assay; n = 7; 3 F and 4 M	Changes in FGCM concentration with time	Mean FGCM decreased initially to 17% (2 h p.d) with subsequent decrease until 50-55% of the original level (8 h p.d), after which it remained stable	Shutt et al. (2012)
Iberian lynx	*lynx pardinus*	Progestin EIA (antibody - monoclonal Pregnane CL425), estrogen (antibody - polyclonal E_2-R4972) testosterone (polyclonalR-156/7) - C Munro, UC Davis USA; n = 7; 11 F and 6 M	Changes in levels of faecal progestin metabolite, faecal estrogen metabolite and faecal testosterone metabolite concentrations with time and season	Faecal progestin metabolite, faecal estrogen metabolite and faecal testosterone metabolite concentration remained stable for 7 d after p.d. The concentration of these metabolites varied significantly with season, with least variation in autumn and winter months.	Abáigar et al. (2010)
White-tailed deer	*Odocoileus virginianus*	[^{125}I] corticosterone RIA; n = 2; 2 F	Changes in levels of faecal glucorticoid metabolite concentrations with varying stimulated conditions (room temperature (20C), high heat (38C), alternating high heat and room temperature cycle, alternating freezing (-20C) and room temperature, stimulated rainfall (0.85cm every alternate day)	Average absolute change (%) in mean faecal glucocorticoid metabolite concentration subjected to five treatment groups showed that stimulated rainfall treatment caused the most notable and consistent changes in FGM concentrations from pre-treatment to 5 d p.d. Most variability in mean FGM concentration for other treatment regimens occurred during the first 24 h with less variability observed across the later days of treatment.	Washburn and Millspaugh (2002)
Baboons	*Pappio spp.*	Corticosterone ^{125}IRIA kit (catalogue 07-120102, ICN Diagnostics, CostaMesa, CA); n = 25; 25 F	Changes in fE_2, fP_4, fT and fGC concentrations with time	fE_2, fP_4 and fT concentration levels did not vary much with time. But fGC levels varied significantly with time delay to freezing and a gradual decrease (9.3% over 30 d) in fGC level is observed. over time.	Beehner and Whitten (2004)

Abbreviations used: CM- corticosterone metabolites; FGM – faecal glucocorticoid metabolites; GCM – glucocorticoid metabolites; FCM – faecal cortisol metabolites; fGCM – faecal glucocorticoid metabolites; EIA – enzyme immuno assay; RIA – radio immunoassay; fE_2 – faecal estradiol; fP_4 – faecal progesterone; fT- faecal testosterone; fGC – faecal glucocorticoids; F – Female; M- Male; n – number of animals used for the study; p.d – post defecation

as sarcoptic mange apart from other stressors like habitat destruction, human interference and vehicle collisions in the wild (Old et al., 2017, Roger et al., 2011, Triggs, 2009). Bare-nosed wombats are also large, fossorial and nocturnal, hence a non-invasive FCM investigation of these animals is ideal for monitoring their physiological status 'remotely' under field conditions.

The present study has the following aims (1) to validate two different EIA techniques (an 'in-house' protocol and a commercial kit) for quantifying FCM in voided faeces of captive bare-nosed wombats (2) to evaluate the decay rate of FCMs exposed to natural conditions during autumn in fresh faecal samples collected from captive bare-nosed wombat.

3.3. Material & Methods

3.3.1 Study site and animals

Faecal samples were collected from adult captive bare-nosed wombats housed at a wildlife park in NSW, Australia. The park had three female wombats (5.5 years, 2 years, and 4 years old) and two male wombats (3 years, and 2 years old). All wombats were kept in separate enclosures, furnished with native grass, logs and burrows. The diet of these wombats mainly consisted of mixed native grasses. The wombats were exposed to humans during daily husbandry and visitor observations. Routine health examination was conducted once a week.

3.3.2 Faecal collection method

Fresh faeces (<12 h) were collected consecutively for two weeks from wombats housed in different enclosures at the park between late April 2017 and early May 2017 (autumn). The faecal samples were collected in labelled sealed bags. The freshness of the samples was assessed by judging the colour, odour, and by visually assessing the moistness of the faeces.

Furthermore, the enclosures were cleaned regularly, ensuring that the faecal samples were voided the night before the day of sample collection. The samples were manually crushed and homogenised inside their respective bags to reduce intra-sample variation. To minimise diurnal variation, all samples were collected between 8:00 am to 9:00 am causing no disturbance to the animal. Additional information such as age, sex, pouch status and diet of the animal were obtained from the keepers. All wombats were clinically healthy, and the females had no pouch young at the time of sample collection.

3.3.3 Experimental Design

Upon collection, on Day 0, each sample was divided into six sub-samples. A small portion (2 g) of each sample was then immediately frozen at -20^{o}C in labelled sealed bags. There was one control sub-sample per wombat sample which provided pre-environmental exposure baseline FCM data. The rest of the five sub-sample aliquots for each wombat sample were then transferred to five separate Petri-dishes (15 x 100 mm) and kept outdoors for five days. All the samples were uniformly exposed to ambient conditions and subsequently frozen at -20^{o}C in separate sealed bags. Daily weather observations were taken from the Bureau of Meteorology, Government of Australia (see http://www.bom.gov.au/).

3.3.4 Faecal hormone extractions

The extraction protocol followed the method as discussed in Wielebnowski et al. (2002) and Narayan et al. (2013). Briefly, the samples were first lyophilized for 24 h in a freeze drier (Edwards Freeze Dryer Modulyo K4) and then heated at 80^{o}C in 1 mL of 90% ethanol for 10 min in a water bath (Julabo TW20). Sample extracts were dried under warm air in a fume cupboard for 48 h. The dried samples adhering to the walls of the microcentrifuge tube were

reconstituted in 1 mL of assay buffer solution (39mM $NaH_2PO_4.H_2O$, 61mM $NaHPO_4$, 0.1% bovine serum albumin and 15mM NaCl, pH 7.0) to separate the solids from the liquid portion, the tubes were then centrifuged at 10,000 rpm for 10 min and the supernatant (1 mL) collected in new tubes.

3.3.5 Faecal cortisol enzyme immunoassay

FCM was quantified by employing two protocols – an 'in-house' and a commercial kit EIA. The 'in-house' EIA followed the methods as detailed in Narayan et al. (2013). Briefly, concentrations of FCM were conducted using a cortisol EIA (R4866; C. Munro, UC Davis, USA; 1:15000 working dilution), horseradish peroxidase bound cortisol label (1:80000 working dilution) and standard solutions of cortisol (1.56-400 pg/well). Samples were assayed on Nunc MaxiSorp™ plates (Sigma-Aldrich; 96 wells). For each EIA, 50 µL of the antibody was diluted to the required concentration (1:15,000) using a coating buffer (50 mmol/L bicarbonate buffer, pH 9.6) to coat the EIA plates. These coated plates were then incubated for ≥ 12 h at 4ºC. Excess unbound antibodies were rinsed with buffered saline containing 0.5 ml/L Tween 20 using an automated plate washer (BioTek ELx50™).

Quantification of extracted FCM was also conducted using a commercial kit (Detect X ® Cortisol Enzyme Immuno Assay Ann Arbor ®, MI, USA). The kit had all the essential reagents including cortisol standard #C040, anti-mouse monoclonal cortisol antibody #C041, cortisol conjugate #C039, assay buffer #X053, wash buffer #X007, TMB substrate #X019, stop solution #X020 and clear coated 96 well plates # X011 (coated with anti-mouse IgG). The protocol followed is detailed in the manufacturer's instructions provided with the kit.

Plates were read using an EL800 (BioTek™) microplate reader (450nm, reference 630nm). The following cross-reactivities are noted: for the 'in-house method' - 100% with cortisol, and

less than 10% with other steroids tested; for the EIA kit - 100% with cortisol, 18.8% with dexamethasone, 7.8% with prednisone (1-Dehydrocortisol), 1.2% corticosterone, 1.2% cortisone, <0.1% with progesterone, <0.1% with estradiol, <0.1% with cortisol 21-glucuronide. Faecal extracts were diluted based on the 50% binding point on the parallelism curve (1:10 for 'in-house' EIA and 1:1.75 for kit EIA). Intra-assay and inter-assay coefficients of variation (CV%) and assay sensitivity based on the analysis of high and low controls were calculated. Sensitivity was evaluated based on 85-90% specific minimum binding. FCM levels were expressed as net dry weight (ng/g) of the faeces.

The data obtained from week 1 samples were used to determine the decay rate of FCMs in bare-nosed wombats while the data obtained from week 2 samples was used to compare FCM levels between the two weeks. The data were analysed accordingly to avoid pseudo replication of data, since samples were collected from the same bare-nosed wombat individuals on day 0 of both weeks.

3.3.6 Statistical Analysis

Statistical tool pack SPSS (ver. 25) and Microsoft® Excel® (2016) Data Analysis tools were used to analyse the data and to generate graphs. For all tests performed the *p*-value was considered significant at $p < 0.05$, FCM considered the dependent variable and time as the independent variable. The data was tested for normal distribution and sphericity using Shapiro-Wilk statistics and Mauchly's sphericity test before performing a repeated measures ANOVA using SPSS. The data did not pass the normality assumption and therefore a nonparametric Friedman test was used to test the effect of time on FCM levels. A Spearman correlation test was run to assess the relationship between CV% in mean daily FCM (combined data of all bare-nosed wombats) with the environmental data.

Samples collected on day 0 of week 1 and week 2 (i.e., with minimal exposure to natural weathering or control samples) can be considered as representative of nominal baseline FCM values. A paired sample t-test was conducted to determine if there was a difference in baseline FCM concentration between week 1 and week 2 of sample collection.

3.4. Results

Assay validation tests resulted in parallel displacement curves between serial dilutions of pooled faecal extracts and cortisol standard (Fig.3.1). For the 'in-house' EIA, the correlation coefficient of the parallelism test was $R^2 = 0.95$, $p < 0.05$. Assay sensitivity was noted as 1.64 pg/well. The inter-assay CV% value was 2.73% at a binding point of 70% and the average intra-assay CV% was 2.3% at a binding point of 50%. For the EIA kit, the correlation coefficient of parallelism test was $R^2 = 0.98$, $p < 0.05$. Assay sensitivity was observed as 31.57 pg/well. The inter-assay CV% was 0.32% at 70% binding point and the mean intra-assay CV% was 2.99 at 50% binding point.

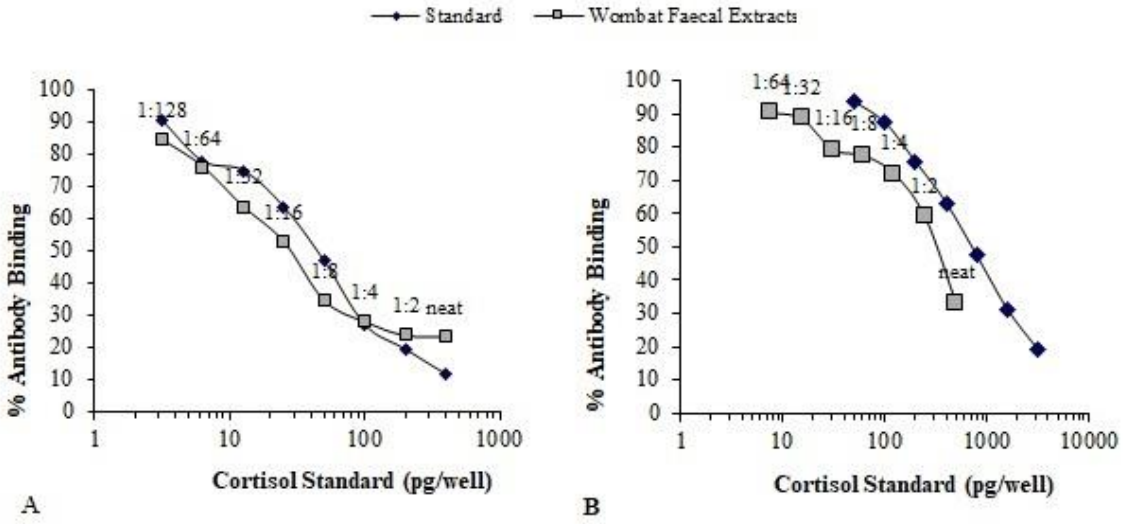

Figure 3.1: Parallelism between standard curve and serial dilutions of wombat faecal extract: graph A – 'in-house' EIA and graph B – kit EIA. A significant relationship was observed in the amounts of antibody bound to cortisol between the wombat faecal extract and the standard solutions created from the synthetic stock ($R^2 = 0.95$, $p < 0.05$ ('in-house' EIA); $R^2 = 0.98$, $p < 0.05$ (kit EIA)).

The results from both EIA techniques employed were similar. Time of exposure to the environment had an insignificant effect on the decay rate of FCM in bare-nosed wombat faecal

samples. The Friedman's test results indicated that FCM concentration did not differ significantly between time 0 h, 24 h, 48 h, 72 h, 96 h and 120 h ($\chi^2(5) = 1.57$, $p = 0.90$ ('in-house' EIA); $\chi^2(5) = 7.90$, $p = 0.16$ (kit EIA)). Since the Friedman's test was not statistically significant, post hoc tests were not performed to determine where the significant differences existed. On comparing the results between the 'in-house' EIA and EIA kit, it was noted that over the 120 h of exposure to natural weathering, the mean FCM value was not observed to vary significantly. However, the highest mean FCM value was recorded at 48 h (29.94 ng/g of dry weight) and the lowest mean FCM value was recorded at 96 h (25.39 ng/g of dry weight) in case of the 'in-house' EIA, whereas the highest mean FCM value was noted at 24 h (21.27 ng/g of dry weight) and the lowest mean FCM value was observed at 48 h (15.33 ng/g of dry weight) for the kit EIA. (Fig. 3.2).

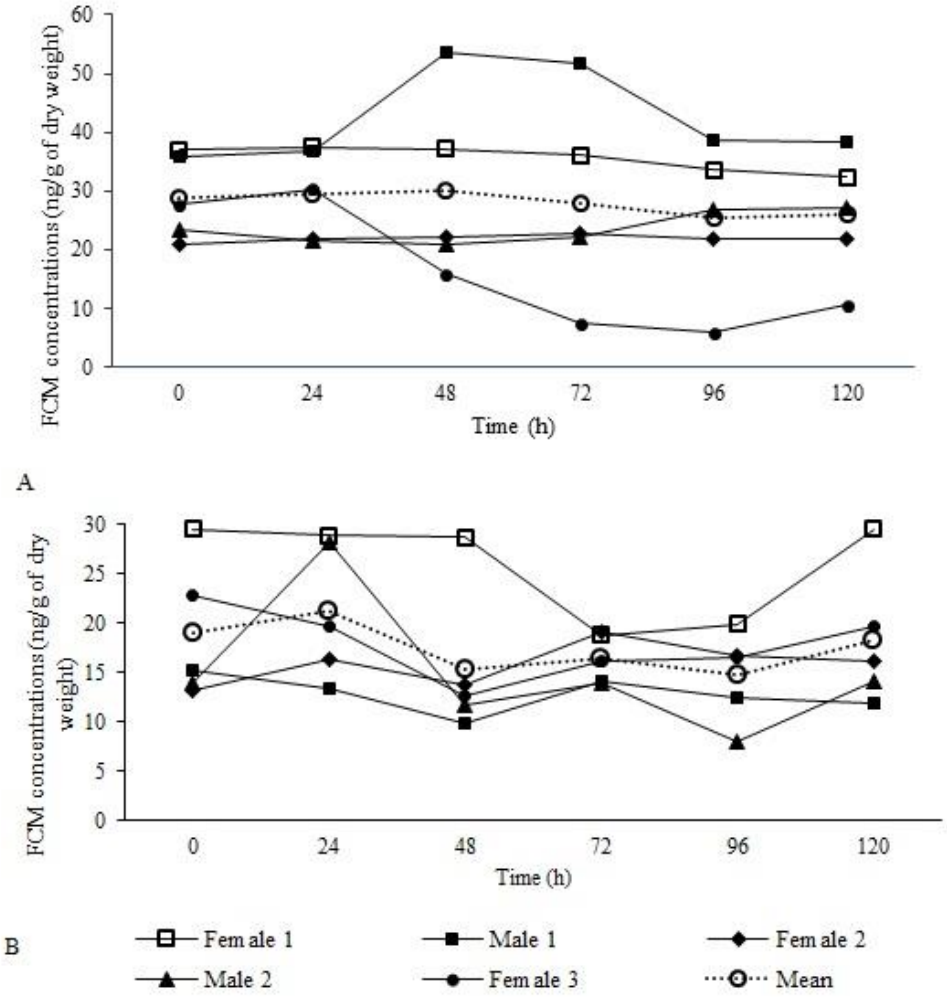

Figure 3.2: Change in faecal cortisol metabolite (FCM) concentrations in samples of bare-nosed wombat samples (n = 5), measured at time intervals 0 and 120 h post-defecation: graph A – 'in-house' EIA and graph B – kit EIA. Symbols represent FCM concentration of each individual bare-nosed wombat and the mean FCM concentration of time (0 h, 24 h, 48 h, 72 h, 96 h and 120 h) for all bare-nosed wombats. All data used in this graph are from samples collected on day 0 of week 1. FCM concentration is expressed as ng/g of dry faecal weight.

A comparison of baseline FCM values between the two weeks showed that there was a significant difference of baseline FCM values between week 1 baseline FCM value and week 2 baseline FCM values (t (4) = 5.78, p = 0.004 (inhouse EIA); t (4) = 3.29, p = 0.03 (kit EIA)).

The paired sample statistics revealed that the mean baseline FCM on day 0 of week 1 (28.97 ng/g ('in-house' EIA) and 18.93 ng/g (kit EIA) was significantly greater than the mean baseline FCM on day 0 of week 2 of 5.53 ng/g ('in-house' EIA) and 11.57 ng/g (kit EIA) (Fig. 3.3).

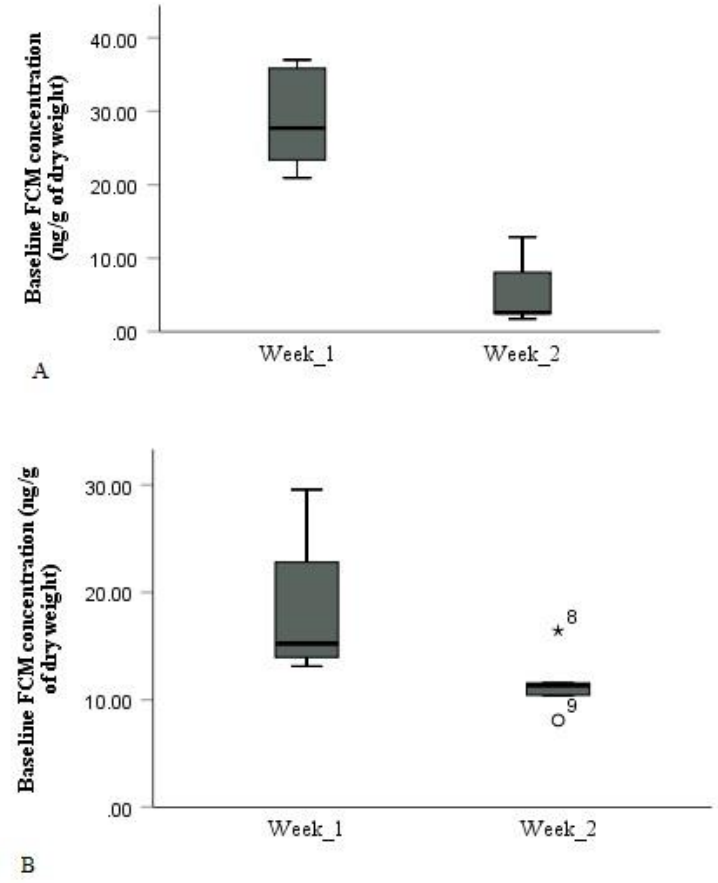

Figure 3.3: Simple box plot representation of baseline FCM values (ng/g) of week 1 (day 0) and week 2 (day 0) samples: graph A – 'in-house' EIA and graph B – kit EIA. Each box representing the upper (75%) and the lower (25%) quartiles and the middle bar representing the median value, while the whiskers mark the highest and the lowest FCM (ng/g) values.

The average maximum temperature during the treatment period was 22.1^{0}C and the average minimum temperature was 8.6°C, whereas the average rainfall was 0.16mm. Spearman correlation indicated no significant relationship between CV % FCM and weather data (p >

0.05) for all correlation comparisons between mean daily CV% and rainfall, minimum and maximum temperature (Fig. 3.4).

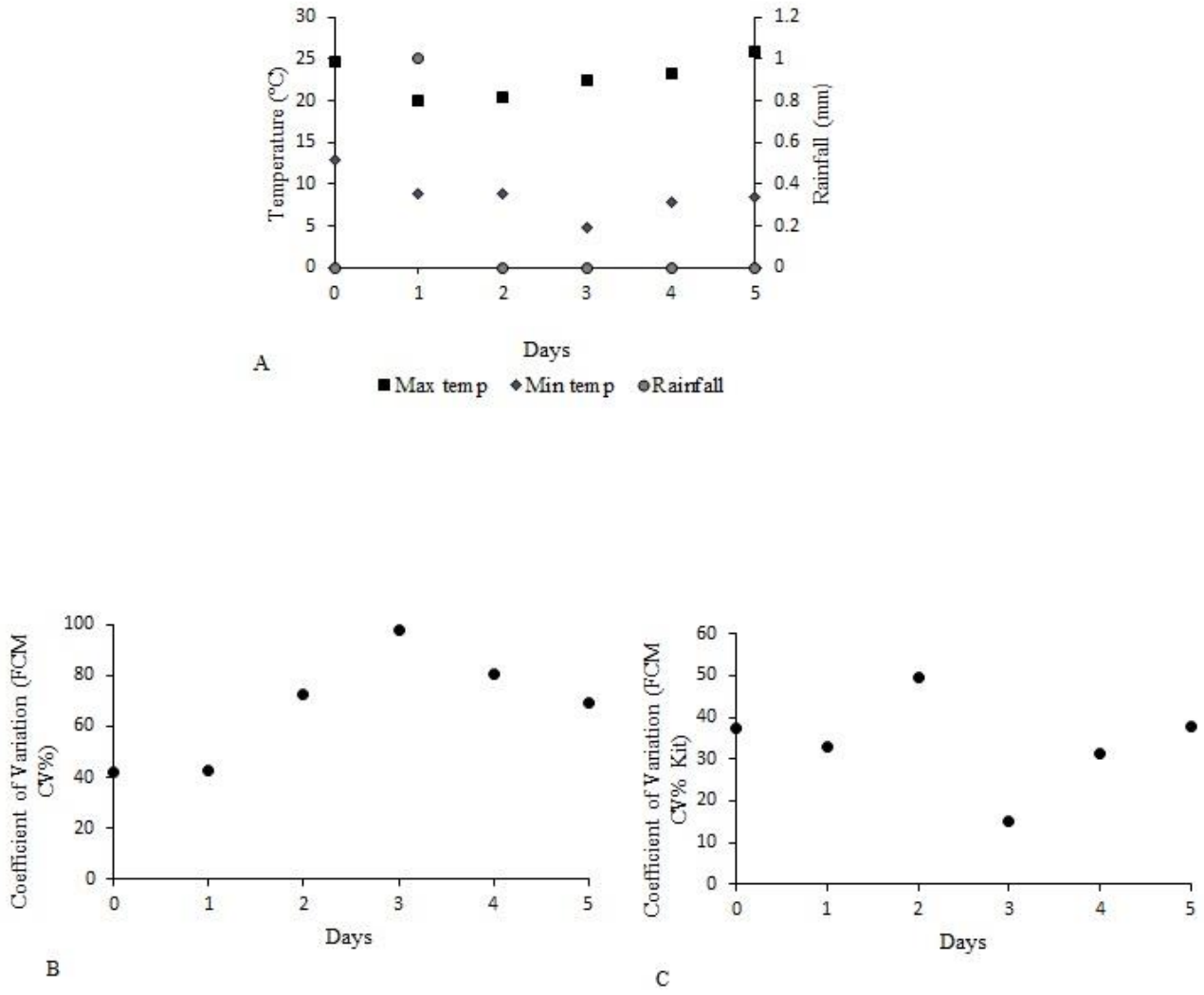

Figure 3.4: Scatterplot representation: A - showing relationships between rainfall, maximum temperature (Tmax), minimum temperature (Tmin), B - coefficient of variation (CV%) of FCM ('in-house' EIA) and C – CV% of FCM (kit EIA). The estimation of CVs was conducted by adding the FCM data of all wombats (of both sexes) for each sampling day.

3.5. Discussion

The results from our study indicated that the mean FCM concentration of bare-nosed wombats remains stable for 120 h (5 days). Therefore, in field conditions collection of fresh faecal samples from bare-nosed wombats during autumn will give reliable FCM EIA results. Standardized protocols to quantify stress in faecal samples of wild animals are limited in the literature. Recent studies have validated FCM analysis in southern hairy-nosed wombat (*Lasiorhinus latifrons*) such as Du et al. (2017), however our study was the first to validate two FCM EIA techniques for bare-nosed wombats and provides support that both of these techniques can be used to monitor adrenocortical activity in wild bare-nosed wombats.

Validation of a FCM EIA technique in any animal is necessary prior to actual experimentation (Touma and Palme, 2005). FCMs are formed after modification of the native hormones in the gut by intestinal bacteria (Palme et al., 2005). In the gut, glucuronide and sulphate moieties are attached to the actual hormonal structures to increase solubility into biological fluids and thus form respective metabolites (Brown et al., 2004). Moreover, the hormone metabolites in faeces is a mixture of different conjugated metabolites (Goymann, 2012) and thus the binding of FCM to monoclonal and polyclonal antibodies designed against native steroids could be less sensitive. Additionally, individuals differing in intestinal bacteria have a varied hormone metabolism. Järvenpää et al. (1980) reported different types of bacteria can metabolize steroid hormones into different metabolites. Therefore, the type of hormone metabolite formed, and their relative composition is dependent on the diversity of bacteria present in the gut. Future research should test other available commercial EIAs to determine the most robust EIA for testing non-invasive steroid profiles in wombats.

Researchers should keep in mind that environmental and animal-related factors (See Fig. 3.5) such as sex of the subject animals, their diet, the ambient temperature, intestinal bacterial composition (Goymann, 2012), their seasonal and daily rhythm, body condition and sample

mass, sample age and preservation conditions (Khan et al., 2002, Millspaugh and Washburn, 2004) can have an effect on the decay rate of FCM. Therefore, the authors recommend performing a biological validation in addition to biochemical validations. For example, the activity of the adrenal cortex in response to induced stress was shown by Dehnhard et al. (2001) on roe deer (*Capreolus capreolus*) where the animals were subjected to an ACTH challenge, a Dex administration and lastly to the effects of a long-acting tranquilizer. This study was able to demonstrate how an ACTH challenge stimulates and Dex administration suppresses adrenocortical activity. Although our study did not incorporate this validation technique in our experimental design, we recommend the use of at least one of these techniques in future to clearly demonstrate the potential of an assay to monitor changes in cortisol secretion in the presence of a stressor.

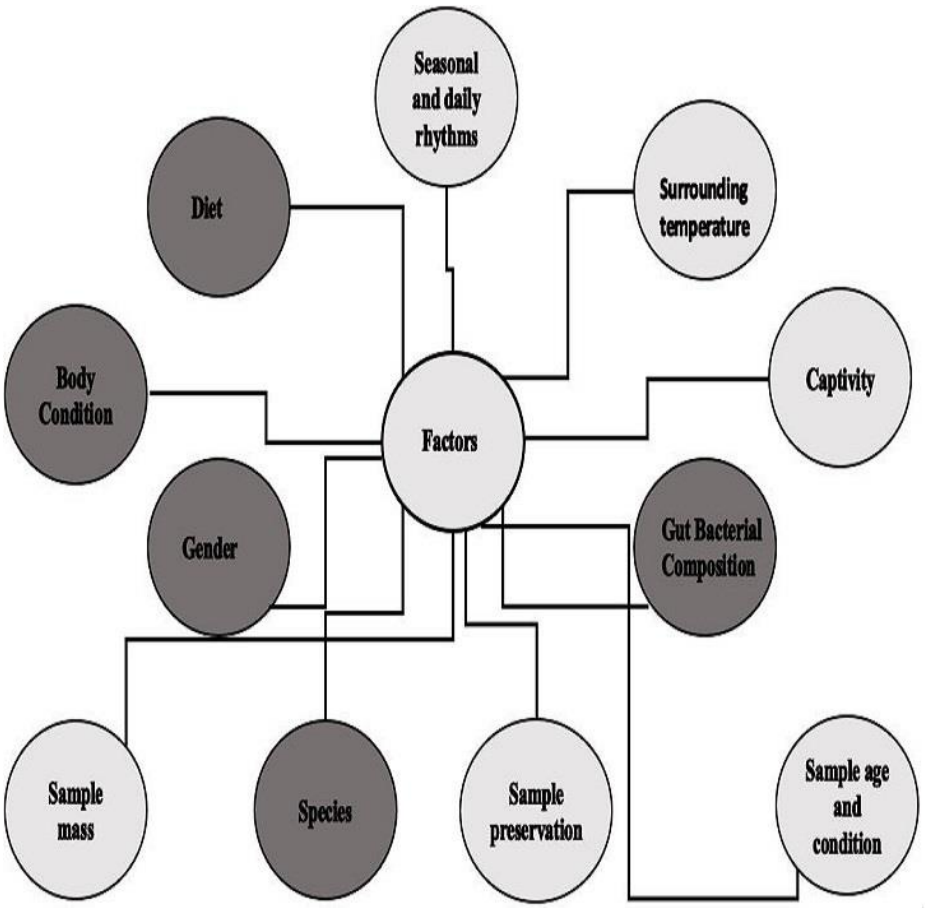

Figure 3.5: Factors to be considered when inferring results of stress analysis. All the factors mentioned influence the baseline FCM level of an individual. These factors act independently and simultaneously with each other. For details see Goymann 2012; Khan et al.2002; Millspaugh and Washburn 2004. The factors in grey circles denote the animal related factors while the factors in white circles denote environment related factors.

Furthermore, we would like to emphasise that the above decay rate finding of cortisol metabolites in faecal samples of captive bare-nosed wombats was performed in autumn months in Sydney, NSW, Australia. Results may vary for samples collected in summer or winter when the precipitation and humidity levels differ.

In our study, a difference of baseline FCM levels between week 1 samples and week 2 samples was noted (Fig. 3). Since other factors including diet and ambient temperature of the captive bare-nosed wombats were similar across the two weeks, one factor that may be responsible for the FCM level differences may be the number of visitors in the park. The sampling for week 1 was conducted at the end of April and the sampling for week 2 was conducted at the beginning of May. More visitors were expected in April compared to May due to school holidays occurring at that time. A rise in the number of visitors may have led to increased FCM levels, as observed in captive male koalas at a Sydney zoo (Webster et al., 2017). Therefore, an increase in human presence in the week prior to the start of the study may have triggered an increase of cortisol metabolites in the scat samples initially collected.

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3.6. Conclusion

This study has reported successful validation and application of two methods for monitoring FCM in bare-nosed wombats via two different cortisol EIAs. Our study confirms collecting wild bare-nosed wombat faecal samples within 12 h -120 h after deposition can be used to obtain reliable FCM EIA results. However, given the variables likely to occur in other seasons, the authors recommend collecting fresh samples immediately after defecation to warrant accurate EIA results.

3.7 Acknowledgements

The authors thank Featherdale Wildlife Park, Sydney, for providing the bare-nosed wombat scat samples and Mr. Mark Emanuel for providing technical assistance. This project is supported by research funds allocated by the WSU SSH to CS for her MPhil research and donations provided to Wombat Giving (JO). Procedures were approved by the Western Sydney

University Animal Care and Ethics Committee (Protocol number: A12033) and WSU Biosafety and Radiation Safety Committee (Protocol number: B10524).

Declaration of Interest

The authors declare no conflict of interest.

3.8 References

Abáigar, T., Domené, M.A., Palomares, F., 2010. Effects of fecal age and seasonality on steroid hormone concentration as a reproductive parameter in field studies. Eur. J. Wildl. Res. 56 781-787. https://hal.archives-ouvertes.fr/hal-00580177/document.

Beehner, J.C., Whitten, P.L., 2004. Modifications of a field method for fecal steroid analysis in baboons. Physiol. Behav. 82 269-277. https://doi.org/10.1016/j.physbeh.2004.03.012.

Brown, J., Walker, S., Steinman, K., 2004. Endocrine manual for the reproductive assessment of domestic and non-domestic species, Endocrine Research Laboratory, Conservation and Research Center, National Zoological Park, Smithsonian Institution, Virginia.

Cockrem, J., Ishii, S., 1999. Environmental and conservation Endocrinology: Conservation Endocrinology - A New Field Of Comparative Endocrinology In: Kwon, H., Joss, J., Ishii, S., (Eds.), Recent Progress in Molecular and Comparative Endocrinology, Kwangju, Korea, pp. 413-418.

Dantzer, B., Fletcher, Q.E., Boonstra, R., Sheriff, M.J., 2014. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? Conserv. Physiol. 2. https://doi.org/10.1093/conphys/cou023.

Dehnhard, M., Clauss, M., Lechner-Doll, M., Meyer, H., Palme, R., 2001. Noninvasive monitoring of adrenocortical activity in roe deer (*Capreolus capreolus*) by measurement of fecal cortisol metabolites. Gen. Comp. Endocrinol. 123 111-120. https://doi.org/10.1006/gcen.2001.7656.

Descovich, K.A., Lisle, A.T., Johnston, S., Keeley, T., Phillips, C.J., 2012. Intrasample variation and the effect of storage delay on faecal metabolite concentrations in the southern hairy-nosed wombat (*Lasiorhinus latifrons*). Aust. Mammal. 34 217-222. https://doi.org/10.1071/AM11047.

Du, Z., Keeley, T., Janssen, T., Nicolson, V., Johnston, S., 2017. Measurement of testosterone and cortisol metabolites and luteinising hormone in captive southern hairy-nosed wombat (*Lasiorhinus latifrons*) urine. Gen. Comp. Endocrinol. 250 70-79. https://doi.org/10.1016/j.ygcen.2017.06.001.

Evans, N., Narayan, E.J., Hero, J.-M., 2013. Effects of natural weathering conditions on faecal cortisol metabolite measurements in the greater bilby (*Macrotis lagotis*). Aust. J. Zool. 61 351-356. https://doi.org/10.1071/ZO13032.

Goymann, W., 2012. On the use of non-invasive hormone research in uncontrolled, natural environments: the problem with sex, diet, metabolic rate and the individual. Methods Ecol. Evol. 3 757-765. https://doi.org/10.1111/j.2041-210X.2012.00203.x.

Järvenpää, P., Kosunen, T., Fotsis, T., Adlercreutz, H., 1980. In vitro metabolism of estrogens by isolated intestinal micro-organisms and by human faecal microflora. J. Steroid Biochem. 13 345-349. https://doi.org/10.1016/0022-4731(80)90014-X.

Khan, M., Altmann, J., Isani, S., Yu, J., 2002. A matter of time: evaluating the storage of fecal samples for steroid analysis. Gen. Comp. Endocrinol. 128 57-64. https://doi.org/10.1016/S0016-6480(02)00063-1.

Kindermann, C., Narayan, E.J., Hero, J.-M., 2017. Does physiological response to disease incur cost to reproductive ecology in a sexually dichromatic amphibian species? Comp. Biochem. Physiol. A Mol. Integr. Physiol. 203 220-226. http://www.sciencedirect.com/science/article/pii/S1095643316302227.

Mesa-Cruz, J.B., Brown, J.L., Kelly, M.J., 2014. Effects of natural environmental conditions on faecal glucocorticoid metabolite concentrations in jaguars (*Panthera onca*) in Belize. Conserv. Physiol. 2. https://doi.org/10.1093/conphys/cou039.

Millspaugh, J.J., Washburn, B.E., 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. Gen. Comp. Endocrinol. 138 189-199. https://doi.org/10.1016/j.ygcen.2004.07.002.

Narayan, E.J., Webster, K., Nicolson, V., Mucci, A., Hero, J.-M., 2013. Non-invasive evaluation of physiological stress in an iconic Australian marsupial: the koala (*Phascolarctos cinereus*). Gen. Comp. Endocrinol. 187 39-47. https://doi.org/10.1016/j.ygcen.2013.03.021.

Old, J.M., Sengupta, C., Narayan, E., Wolfenden, J., 2017. Sarcoptic mange in wombats—A review and future research directions. Transbound. Emerg. Dis. 65 399-407. http://dx.doi.org/10.1111/tbed.12770.

Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S., Möstl, E., 2005. Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Ann. N. Y. Acad. Sci. 1040 162-171. https://doi.org/10.1196/annals.1327.021.

Parnell, T., Narayan, E.J., Nicolson, V., Martin-Vegue, P., Mucci, A., Hero, J.-M., 2015. Maximizing the reliability of non-invasive endocrine sampling in the tiger (Panthera tigris): environmental decay and intra-sample variation in faecal glucocorticoid metabolites. Conserv. Physiol. 3. https://doi.org/10.1093/conphys/cov053.

Pellegrini, S., Busso, J., Lèche, A., Marin, R., 2015. Effects of diet, time since defecation, and drying process of the droppings on corticosterone metabolite measurements in Japanese quail. Poult. Sci. 94 1068-1074. https://doi.org/10.3382/ps/pev072.

Rehnus, M., Hackländer, K., Palme, R., 2009. A non-invasive method for measuring glucocorticoid metabolites (GCM) in Mountain hares (*Lepus timidus*). Eur. J. Wildl. Res. 55 615. https://doi.org/10.1007/s10344-009-0297-9.

Rodrigues, M.A., Wittwer, D., Kitchen, D.M., 2015. Measuring stress responses in female Geoffroy's spider monkeys: Validation and the influence of reproductive state. Am. J. Primatol. 77 925-935. https://doi.org/10.1002/ajp.22421.

Roger, E., Laffan, S.W., Ramp, D., 2011. Road impacts a tipping point for wildlife populations in threatened landscapes. Popul. Ecol. 53 215-227. https://doi.org/10.1007/s10144-010-0209-6.

Sheriff, M.J., Dantzer, B., Delehanty, B., Palme, R., Boonstra, R., 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia. 166 869-887. https://doi.org/10.1007/s00442-011-1943-y.

Shutt, K., Setchell, J.M., Heistermann, M., 2012. Non-invasive monitoring of physiological stress in the Western lowland gorilla (*Gorilla gorilla gorilla*): validation of a fecal glucocorticoid assay and methods for practical application in the field. Gen. Comp. Endocrinol. 179 167-177. https://doi.org/10.1016/j.ygcen.2012.08.008.

Terio, K.A., Brown, J.L., Moreland, R., Munson, L., 2002. Comparison of different drying and storage methods on quantifiable concentrations of fecal steroids in the cheetah. Zoo Biol. 21 215-222. https://doi.org/10.1016/j.ygcen.2012.08.008.

Touma, C., Palme, R., 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Ann. N. Y. Acad. Sci. 1046 54-74. https://doi.org/10.1196/annals.1343.006.

Triggs, B., 2009. Wombats, 2nd ed. CSIRO PUBLISHING Collingwood, Vic.

Vaz, J., Narayan, E.J., Kumar, R.D., Thenmozhi, K., Thiyagesan, K., Baskaran, N., 2017. Prevalence and determinants of stereotypic behaviours and physiological stress among tigers and leopards in Indian zoos. PLoS One. 12 e0174711. https://doi.org/10.1371/journal.pone.0174711.

Washburn, B.E., Millspaugh, J.J., 2002. Effects of simulated environmental conditions on glucocorticoid metabolite measurements in white-tailed deer feces. Gen. Comp. Endocrinol. 127 217-222. https://doi.org/10.1016/S0016-6480(02)00056-4.

Webber, J.T., Henley, M.D., Pretorius, Y., Somers, M.J., Ganswindt, A., 2018. Changes in African Elephant (*Loxodonta africana*) faecal steroid concentrations post-defaecation. Bothalia. 48 8. https://doi.org/10.4102/abc.v48i2.2312.

Webster, K., Narayan, E., de Vos, N., 2017. Fecal glucocorticoid metabolite response of captive koalas (*Phascolarctos cinereus*) to visitor encounters. Gen. Comp. Endocrinol. 244 157-163. http://www.sciencedirect.com/science/article/pii/S001664801630209X.

Wielebnowski, N.C., Fletchall, N., Carlstead, K., Busso, J.M., Brown, J.L., 2002. Noninvasive assessment of adrenal activity associated with husbandry and behavioral factors in the North American clouded leopard population. Zoo Biol. 21 77-98. https://doi.org/10.1002/zoo.10005

Wikelski, M., Cooke, S.J., 2006. Conservation physiology. Trends Ecol. Evol. 21 38-46. https://doi.org/10.1016/j.tree.2005.10.018.

Wong, E.P., Yon, L., Purcell, R., Walker, S.L., Othman, N., Saaban, S., et al., 2016. Concentrations of faecal glucocorticoid metabolites in Asian elephant's dung are stable for up to 8 h in a tropical environment. Conserv. Physiol. 4. https://doi.org/10.1093/conphys/cow070.

Chapter 4: Evaluating the role of stress and parasite load in sarcoptic mange incidence in bare-nosed wombats (*Vombatus ursinus*) in N.S.W., Australia

4.1 Chapter outline:

Chapter 4 determined the current stress load, endoparasitic load and sarcoptic mange prevalence in bare-nosed wombats located at five different locations in NSW, Australia employing non-invasive techniques. The causal link between sarcoptic mange susceptibility in bare-nosed wombats and stress load and endoparasitic load was evaluated. The degree of this relationship between sarcoptic mange, stress level and endoparasitic load in bare-nosed wombats was further assessed. This study was conducted under the approval of Western Sydney University Animal Care and Ethics Committee (Protocol number: A12033), WSU Biosafety and Radiation Safety Committee (Protocol number: B10524) and NSW National Parks and Wildlife Services (Licence number: S13202).

This paper is jointly co-authored, where I am the primary and corresponding author. I analysed the endoparasitic load present in the bare-nosed wombat faecal samples, extracted faecal cortisol metabolites from bare-nosed wombat faecal samples, performed enzyme-immunoassays on extracted faecal cortisol metabolites, analysed the data and drafted the manuscript. Associate Professor Julie Old is the second author and she conceived the study, supervised the development of the study, supervised the development of the manuscript and provided editorial feedback on the manuscript drafts. Dr Edward Narayan is the third author and he provided feedback on data analysis and supervised the development of the manuscript drafts.

This paper is under review as a publication in International Journal for Parasitology – Parasites and Wildlife and has been formatted as per journal specification.

https://www.journals.elsevier.com/international-journal-for-parasitology-parasites-and-wildlife

4.2. Introduction

Bare-nosed wombat (*Vombatus ursinus*) populations in Australia have been declining over the last few decades (Fraser et al., 2016). Apart from vehicle collisions, anthropogenic habitat fragmentation and climate change, the major factor responsible for depleting bare-nosed wombat populations is sarcoptic mange (Old et al., 2017, Roger et al., 2011, Triggs, 2009).

Sarcoptic mange is a debilitating disease affecting over 104 mammal species globally (Pence and Ueckermann, 2002). Astigmatic mites measuring 213- 504 μm by 162- 420 μm (Arlian, 1989) are reported to burrow through the epidermis of the host causing hypersensitivity reactions (Skerratt, 2003). Bare-nosed wombats severely infested with mites lose hair, become emaciated, and have cracked skin with secondary bacterial infections (Old et al., 2017, Pence and Ueckermann, 2002, Skerratt, 2001). These marsupials can become blind and deaf due to severe and prolonged sarcoptic mange mite infestations around their eyes and ears (Hartley and English, 2005, Triggs, 2009).

The emergence and host expansion of sarcoptic mange in wildlife have raised concerns amongst scientists worldwide (Alasaad et al., 2011, Astorga et al., 2018, Tompkins et al., 2015). In Australia, bare-nosed wombats have been reported to be most vulnerable to sarcoptic mange compared to other wildlife species (Old et al., 2017). Consequently, researchers across Australia have begun to investigate the disease dynamics and reason for increased susceptibility of bare-nosed wombats to sarcoptic mange (Fraser et al., 2016, Hermsen, 2015, Martin et al., 2018). Identifying factors that influence the incidence of sarcoptic mange in wombats can help in designing future management strategies.

Previous studies have indicated the role of gut parasites in shaping overall host fitness (Kutzer and Armitage, 2016). Strongyle and *Strongyloides* nematodes are known to reduce host fitness by causing weight loss, emaciation and reduced reproductive ability (Gulland, 1992, Lello et

al., 2005), which can cause significant stress to the animal. No literature exists which investigates the relationship between gut parasites and sarcoptic mange incidence in bare-nosed wombats. Furthermore, previous studies on fishes (Snieszko, 1974), koalas (*Phascolarctos cinereus*) (Canfield et al., 1991) and on captive marsupials (Thompson et al., 2010) suggest chronic stress (we define this as maladaptation of the stress endocrine system function) can be a contributing factor in infectious disease incidence. Sapolsky, (2002) reported that animals enduring chronic stress can have low immunocompetence, therefore, can be more susceptible to diseases. Moreover, affected animals can have reduced fecundity as has been observed in frogs (*Litoria wilcoxii*) (Kindermann et al., 2017).

Animals cope with changes in their environment by initiating a physiological stress response that is characterised by the activation of hypothalamic-pituitary-adrenal axis (HPA) with the release of glucocorticoids as end products (Sheriff et al., 2011, Wikelski and Cooke, 2006). In fact, long term activation of the HPA can result in adverse effects on overall health. Measurement of stress can reflect an individual's physiological response to combined effects of chronic diseases, anthropogenic habitat fragmentation, climate change and starvation (Hing et al., 2014, Romero, 2004). For the past few decades non-invasive quantification of glucocorticoid metabolites in faeces, urine, saliva and hair as indicators of physiological stress is routine (Dantzer et al., 2014, Sheriff et al., 2011). However, species-specific validations of non-invasive endocrine assessments are mandatory (Schwarzenberger, 2007, Touma and Palme, 2005). The protocol for extraction and quantification of faecal glucocorticoid hormone metabolites (cortisol metabolites) in bare-nosed wombat has been validated, and the decay rate of faecal cortisol metabolites (FCM) and baseline FCM levels has been evaluated (Sengupta et al., Under Review).

Previous research has not addressed the relationship between stress and endoparasitic burden in increased vulnerability to sarcoptic mange in bare-nosed wombats. In this study, we

investigated this research question in the four free-living and one captive bare-nosed wombat population.

4.3. Materials and Methods

4.3.1 Study sites:

Five locations in NSW (four free-living and one captive population) were selected to collect samples and to estimate population density of bare-nosed wombats – Emirates One&Only Wolgan Valley, Newnes (33° 15'S, 150° 10'E), Eagle's Drift, Merriwa (32° 11'S, 150° 05'E), Badger Ground Wildlife Sanctuary, Rylstone (32° 38'S, 149° 58'E), Cedar Creek Wombat Rescue Inc. and Hospital, Cessnock (32°50'S,151°21'E) and Coolagolite (36° 22' 49.029"S 150° 1' 18.354"E), which are hereinafter referred to as Wolgan Valley, Merriwa, Rylstone, Cessnock and Coolagolite study sites respectively in this study (Fig 4.1).

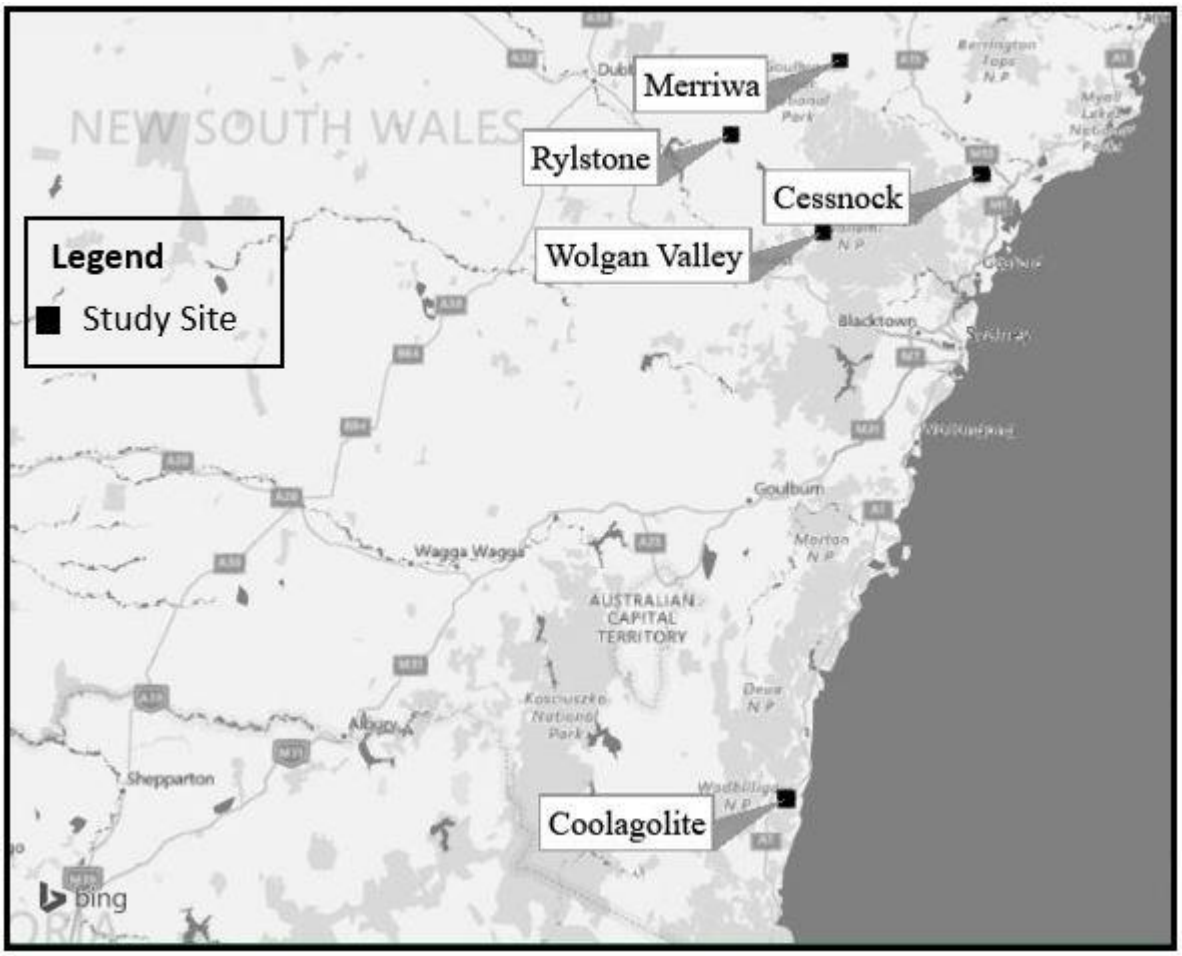

Figure 4.1: Map showing locations of study sites from where bare-nosed wombat faecal samples were collected.

4.3.2 Sample collection:

Faecal samples were collected between 7.00 am - 9.00 am, because bare-nosed wombats are nocturnal (Evans, 2008), and the excretion of parasite eggs and FCM into faeces follows a diurnal rhythm. The samples were collected in sealed plastic bags which were placed into an esky with icepacks and transported to the laboratory on the same day. The samples were weighed and homogenised inside individual sealed bags at the laboratory. One half of each sample was used for stress hormone analysis and the other half was used to determine faecal egg counts (FEC). Samples for hormone analysis were kept at -20ºC until extraction while the samples for estimating the FEC were stored at 4ºC until analysis.

We collected 65 faecal samples from the Wolgan valley (n = 13), Rylstone (n = 9), Merriwa (n = 28), Cessnock (n = 7) Coolagolite (n = 8) between February 2017 and April 2018. All samples were collected during autumn months, except the Coolagolite samples, which were collected during spring.

Captive samples were obtained from Cessnock, and these samples were utilised as the medical history of these animals was known (Table 4.1) hence presumed clinical indicators of stress available. The samples were collected from two female (10-12 months; 7 months) and three male (10 months; 10 months; 12-13 months) bare-nosed wombats.

Table 4.1: Health information of bare-nosed wombats housed in Cedar Creek Wombat Rescue Inc. & Hospiatl during sampling.

Wombat Id	**Clinical Condition**	**Treatment**	**Additional comments**
C285	Symptoms of sarcoptic mange present	Ivermectin injections	Past record of attack by other wombats..
C284	Symptoms of sarcoptic mange present	Ivermectin injections	Mother had extreme sarcoptic mange infestation.
C287	No symptoms of sarcoptic mange; vitamin deficiency	Vitamin injections	Past record of attacks by other wombats.
C286	No symptoms of sarcoptic mange; has dry skin	N. A	
C288/C289	Healthy wombat (had sarcoptic mange 6 months ago)	Ivermectin injections (second round of course)	

4.3.3 Sarcoptic mange prevalence: Spotlighting

Sarcoptic mange prevalence was obtained by conducting spotlighting and utilising the sarcoptic mange scoring system as per Wolfenden and Old, (2012). Spotlighting surveys were conducted only at Wolgan Valley, Merriwa, Coolagolite and Rylstone.

The transects used for the survey were previously surveyed by researchers at Western Sydney University (Hermsen, (2015); Old et al., (unpublished)). Spotlighting was conducted following the method described in Hermsen (2015) and Old et al., (unpublished). Briefly, a 4WD vehicle was driven after dark along these transects at a speed of 5-10km/h. Two volunteers acted as observers who scanned the area for animals utilising 100W halogen spotlights (Powa-Beam: PL-145,12v) and another acted as the "scribe" to note down the location (using a Global Positioning System (GPS)), time of observation, and distance and compass bearing of the wombat from the vehicle. Other parameters such as rainfall, cloud cover, moon phase and wind direction were documented. Additional species observed during spotlighting surveys were recorded as they may be potential reservoirs of sarcoptic mange (Skerratt et al., 1998). During each spotlighting observation of a wombat, the animal was given a mange score (see Wolfenden and Old, (2012)). Mange score 'unknown' was given to wombats which that could not be definitively scored due to visual obstructions. For details on spotlighting area, duration of spotlighting activity on each study site and number of spotlighting surveys conducted for each site see table 4.2. Data of all spotlighting surveys conducted for each study site was combined to be used for statistical analysis.

4.3.4 Estimation of parasite burden:

Samples for FEC were kept at 4ºC and egg counting was conducted within seven days of collection except the samples collected from Merriwa, where FEC was completed within two

months of collection. In the laboratory 2g of each faecal sample were floated in a saturated salt solution for identification of parasites such as helminth eggs and larvae, protozoan oocysts and cysts using the modified McMaster technique (Whitlock, 1948). A Whitlock universal slide was used to count the number of eggs and a mathematical formula to estimate the number of eggs per gram (e.p.g) of faeces as detailed in Corner and Bagust, (1993). Eggs were counted systematically using a CX23 (Olympus, Japan) microscope. Mean e.p.g were calculated for each study site population.

4.3.5 Faecal Cortisol Metabolites (FCM) analysis:

All FCM extractions were carried out within three months of collection. The protocol for extraction is detailed in Sengupta et al., (Under Review). In brief, samples were lyophilised for 24 h before being heated in 1 mL of 90% ethanol at 80^{o}C for 10 min. Then the samples were dried in a fume cupboard and reconstituted in enzyme immunoassay (EIA) buffer solution (39mM $NaH_2PO_4.H_2O$, 61mM $NaHPO_4$, 0.1% bovine serum albumin and 15mM NaCl, pH 7.0).

Quantification of extracted FCM was conducted using Detect X ® Cortisol Enzyme Immuno Assay kit (Arbor Assays, Ann Arbor ®, MI, USA) as per the manufacturer's instructions provided with the kit. The following cross reactivities were reported - 100% with cortisol, 18.8% with dexamethasone, 7.8% with prednisone (1-Dehydrocortisol), 1.2% corticosterone, 1.2% cortisone, <0.1% with progesterone, <0.1% with estradiol, <0.1% with cortisol 21-glucuronide. Plates were read using an EL800 (BioTek^{TM}) microplate reader (450nm, reference 630nm).

4.3.6 Statistical Analysis:

Statistical tool pack IBM® SPSS® (ver. 25) was used to analyse the data and to generate graphs. For all tests performed the *p*-value was considered significant at $p < 0.05$. Tests of normality and homogeneity of equal variances were conducted using Saphiro-Wilk and Levene's statistics respectively. The data for sarcoptic mange incidence, FCM level and the FEC data violated the normality and homogeneity of variances assumption ($p < 0.05$). Consequently, a non-parametric Kruskal-Wallis one-way analysis of variance test was conducted to test the differences between mean sarcoptic mange prevalence, mean FCM level and mean egg counts of the five study locations. For all tests study location was considered as an independent variable.

Scatterplots were constructed and regression lines were fitted to enable an understanding of the association between mean sarcoptic mange incidence, mean FCM and mean FEC. Spearman rank correlation tests were carried out to evaluate the degree of association between variables. The FCM and FEC data were log-transformed for clarity.

4.4. Results

4.4.1 Study sites and Sarcoptic Mange prevalence:

A total of 319 bare-nosed wombats were observed during spotlighting, of which a mean number of 42.42 ± 4.52 S.E., 6 ± 0 and 9 ± 2 S.E. wombats were observed per night at Wolgan Valley, Merriwa and Rylstone (Table 4.2). The spotlighting survey revealed that 73%, 13%, 3% and 1% of bare-nosed wombats were observed with mange score 1, mange score 2, mange score 3 and mange score 4 respectively. In addition, 7.83% of wombats could not be given a mange score due to obstructed visibility. No wombats with mange score 5 were observed in any of the spotlighting survey locations, despite wombats with a sarcoptic mange score 5 being

opportunistically observed during the day. Of the four spotlighting locations the Wolgan Valley recorded the highest mean number of sarcoptic mange affected wombats compared to Rylstone, Merriwa and Coolagolite (Fig 4.2). Kruskal Wallis H statistics found no statistically significant difference ($\chi^2(3) = 5.71$, $p = 0.12$) between the mean number of sarcoptic mange incidences for the four free-range study locations. For details on sarcoptic mange incidence for the Cessnock population see Table 4.1, since spotlighting surveys were not conducted for this study site.

Table 4.2: Details of Spotlighting data

Site Name	No. of Surveys	Spotlighting area(ha)	Mean Duration (min)	Mean wombat/night (±S.E.)	Mean wombats/ha	Mean wombats/h
Wolgan Valley	7	191	164.85	42.42±4.52	1.55	15.44
Merriwa	1	80	112	6±0	0.07	3.12
Rylstone	2	87	68	9±2	0.20	7.94

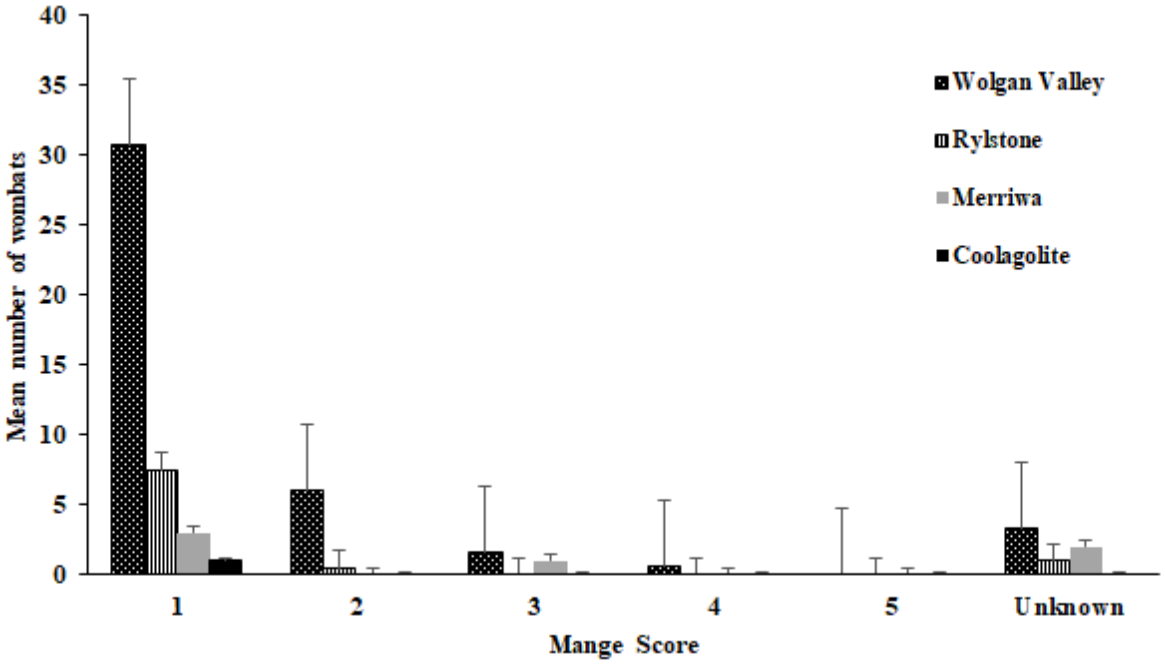

Figure 4.2: Mean sarcoptic mange prevalence as per data collected from spotlighting surveys from the four free-ranging study sites – Wolgan Valley, Rylstone, Merriwa and Coolagolite. Each bar represents the average number of wombats observed on a study site and the error bars represent standard error.

Mange score 1 = no visible signs of sarcoptic mange.

Mange score 2 = Ears and eyes free of mange, small, sparse patches of hair loss; skin appears slightly crusty in these spots, usually on the side of the body.

Mange score 3 = Ears appear normal; area around eyes is beginning to appear crusty; large portions of hair loss on the sides of the body, skin appears crusty in these regions; mange is starting to spread to the limbs; small lesions may be present.

Mange score 4 = Slightly emaciated; ears are thick and crusty, appearing 'cauli-flowered'; eyes are crusty and closed; most of the hair on the sides, limbs and face is lost; skin is very thick and starting to appear blue/grey, lesions likely to be present; still has hair on the top of the body.

Mange score 5 = Extremely emaciated; ears are thickened and crusty, extensively 'cauliflowered'; hair is still present on the head; almost all hair is gone, skin is very crusty and appears blue/grey, and lesions are present; eyes are very crusty and cannot open or close; may be completely deaf and blind, easily approached.

Mange score Unknown = Exact score could not be given to the wombat due to visual obstructions.

4.4.2 Estimation of parasitic burden:

The non-parametric Kruskal Wallis H test revealed a statistically significant effect ($\chi^2(4) = 40.14$, $p = 0.00$), indicating a difference in average e.p.g count between the five study sites. Dunn's pairwise comparisons between groups indicated a significant difference ($p < 0.05$, adjusted using Bonferroni correction) in mean rank e.p.g score between wombat population of Wolgan Valley and Coolagolite, between Wolgan Valley and Merriwa and between Wolgan Valley and Cessnock. The highest mean e.p.g was recorded at the Wolgan Valley (389.23 ± 144.30 S.E.) and the lowest mean e.p.g was recorded at Merriwa (0.71 ± 1.04 S.E.) (Fig 4.3 A). All eggs had distinctive outer cell membranes and inner cellular masses with egg sizes varying between 70µ - 120µ in length and approximately 40µ in breadth (Fig 4.4 A). Viable adult larvae were sited in Merriwa samples (Fig 4.4 B).

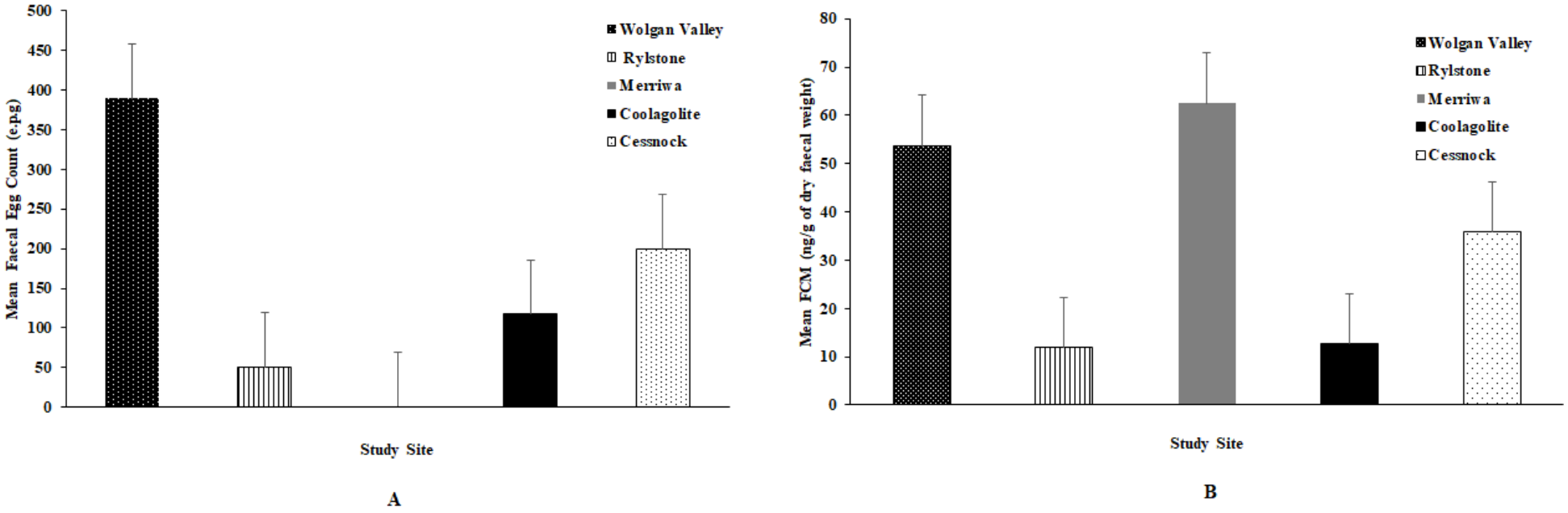

Figure 4.3: A. Bar diagram representing mean strongyle egg count (e.p.g) obtained from the faecal egg count analysis conducted on scat samples collected from five study sites of wild bare-nosed wombats. B. Bar diagram representing the mean faecal cortisol metabolite values (ng/g of dry weight) obtained from the faecal cortisol metabolite enzyme immunoassay conducted on scat samples collected from five study sites of wild bare-nosed wombats. Each bar represents the average faecal egg count of a study site and the error bars represent standard error.

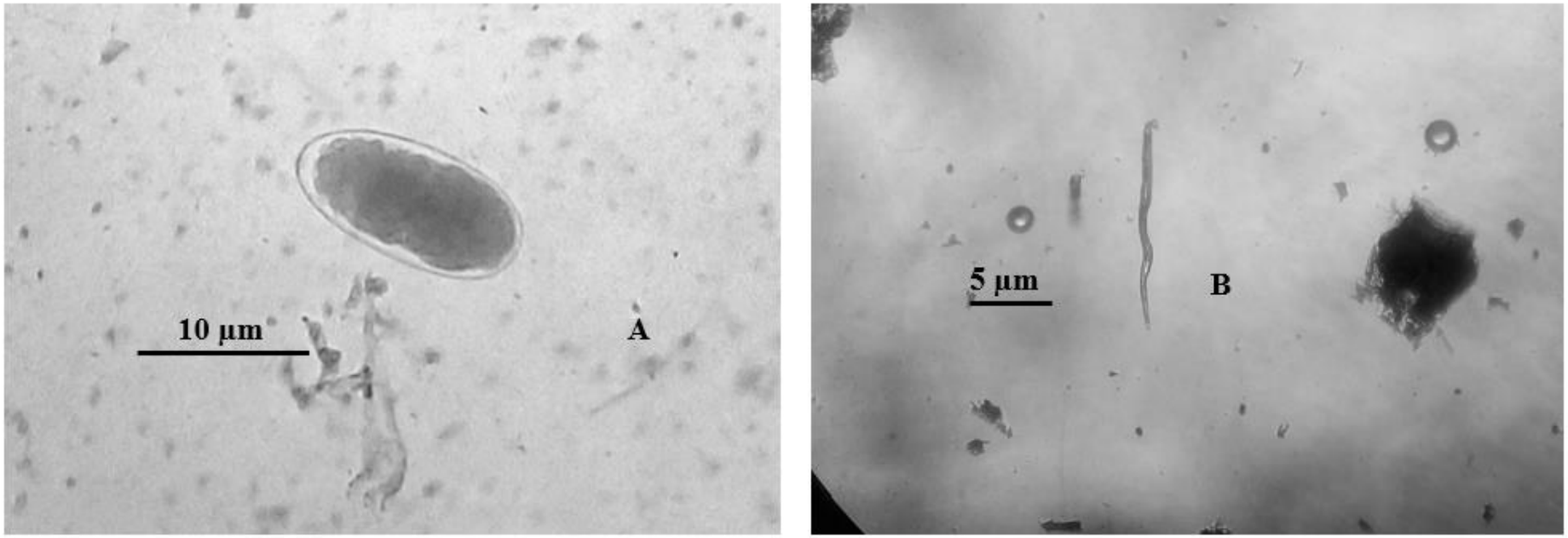

Figure 4.4: A: Strongyle egg; B: Adult larvae

4.4.3 Faecal Cortisol Metabolite Levels:

A non-parametric Kruskal-Wallis H test revealed that there was a significant difference in FCM levels between different study locations ($\chi^2(4) = 14.48$, $p = 0.006$). Dunn's pairwise comparison test indicated a significant difference ($p < 0.05$, adjusted using Bonferroni correction) in mean rank FCM score between wombat population of Coolagolite and Wolgan Valley and between Rylstone and Wolgan Valley. The mean FCM value differed significantly between the study sites with the highest mean FCM observed at Merriwa (62.52 ± 22.05S.E. ng/g of dry weight) and the lowest mean FCM recorded at Rylstone (11.88 ± 3.17S.E. ng/g of dry weight) (Fig 4.3 B). As per Sengupta et al., (Under Review) the baseline FCM level in captive bare-nosed wombats range from 23.95 - 8.55 ng/g of dry faecal weight (the baseline FCM data is the mean of all data obtained at time 0). Our results show that, of the five locations three sites (Wolgan Valley, Merriwa and Cessnock) had bare-nosed wombats with comparatively higher FCM levels than the baseline FCM level reported in Sengupta et al., (Under Review).

Scatterplots and regression fit lines revealed that the relationship between FCM and FEC was linear (Fig 4.5). A strong positive linear association between FCM and FEC for samples from the Wolgan Valley and Coolagolite was observed, whereas a weak positive linear association was observed for Merriwa. In comparison, a strong negative association was observed between FCM and FEC for samples collected from Rylstone and Cessnock. Overall, a Spearman rank correlation test was unable to suggest a statistically significant correlation (r_s (n = 65) = 0.08, $p = 0.50$) between the FCM and FEC data.

Strong positive linear associations were suggested by scatterplots between sarcoptic mange incidence and mean FCM level as well as between mean sarcoptic mange incidence and mean FEC (Fig 4.6). Spearman rank correlation tests reported a strong positive correlation between

mean sarcoptic mange prevalence and mean FCM (r_s (n = 5) = 0.90 p = 0.03)), In contrast, no evidence of significant statistical correlation was indicated between the mean sarcoptic mange prevalence and mean FEC (r_s (n = 5) = 0.10 p = 0.87).

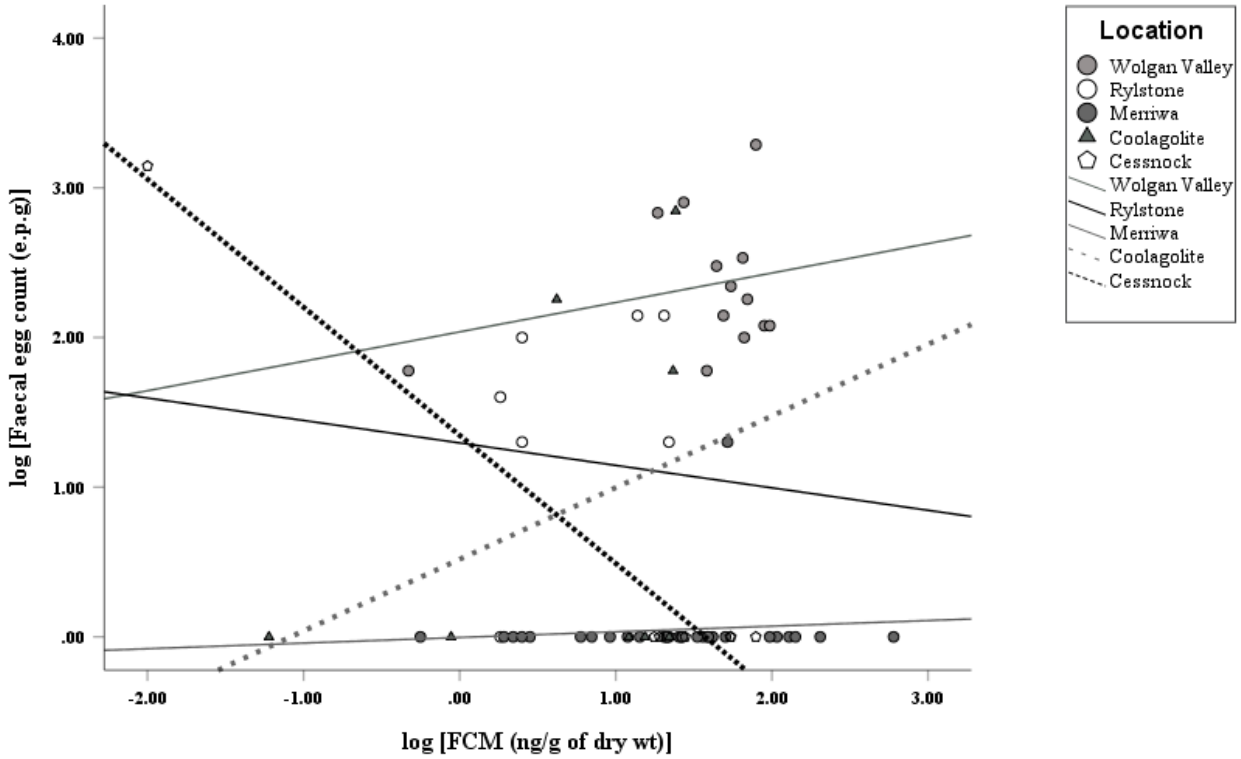

Figure 4.5: Scatterplot and trendlines showing the variation in log-transformed faecal egg count (e.p.g) with log-transformed faecal cortisol metabolite level (ng/g of dry weight) in samples (n = 65) collected from five study sites.

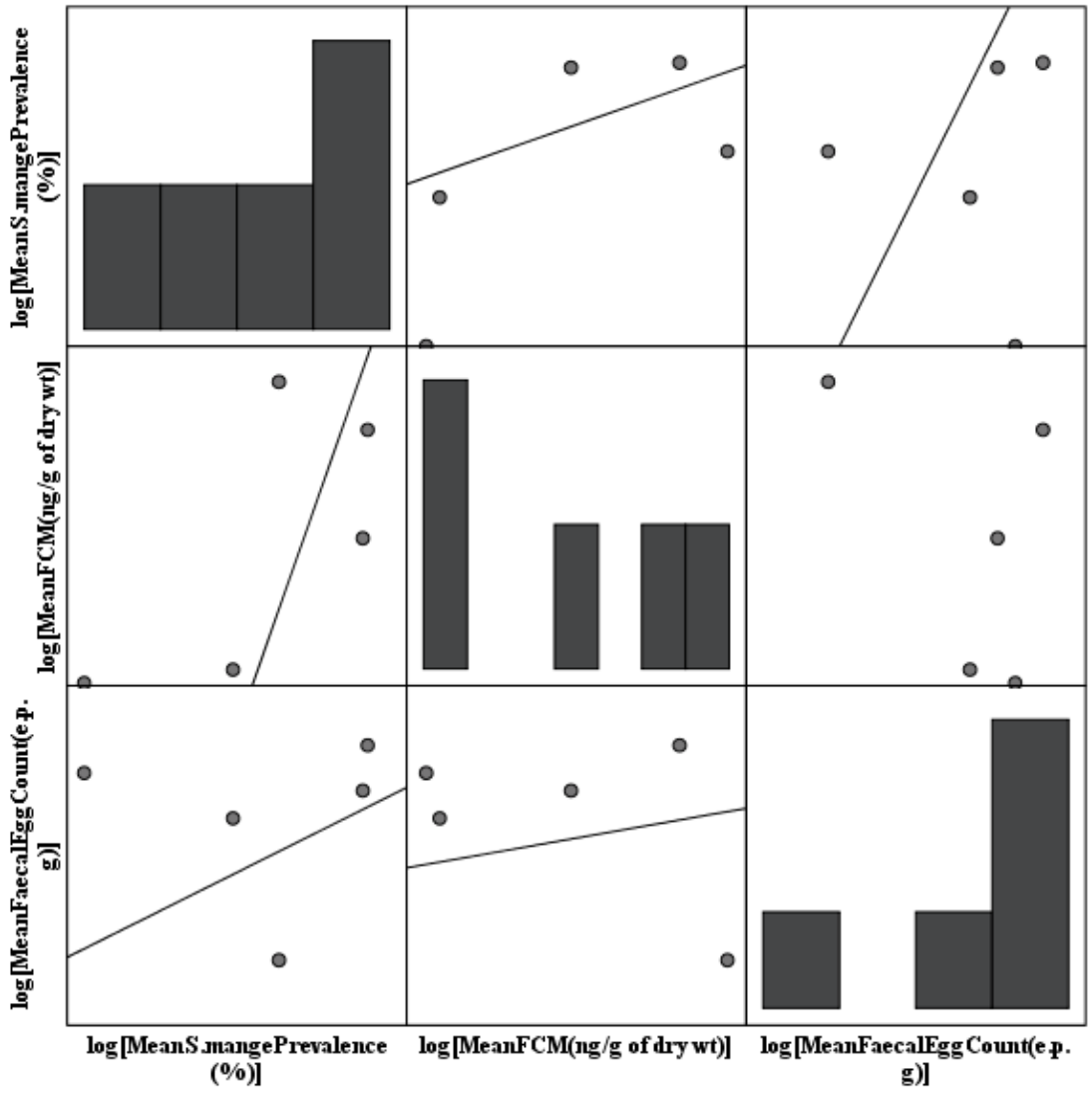

Figure 4.6: Scatterplot matrix and trendlines showing variation in log-transformed mean sarcoptic mange prevalence (%), log-transformed mean faecal cortisol metabolite (ng/g of dry wt) and log transformed mean faecal egg count (e.p.g) in wombat populations (n = 5). Distribution of each variable is shown in diagonal.

4.5. Discussion

Limited studies explored the relationship between gut parasites and stress in mammals (Carlsson et al., 2016, Chapman et al., 2006, Cizauskas et al., 2015, Goldstein et al., 2005, Monello et al., 2010). Furthermore, there is a lack of literature which explores the causal

relationship between stress, gut parasites and sarcoptic mange. To the best of our knowledge, our study is the first to offer an insight into the stress level, helminth burden and sarcoptic mange prevalence of five bare-nosed wombat populations located in NSW, Australia (Table 4.3). Although a strong statistically significant positive correlation has been indicated between mean sarcoptic mange prevalence and mean FCM, readers should interpret the data with caution since the sample size is low. Low sample size has limited our ability to make a definite statistical inference regarding the relationship between mean sarcoptic mange and FEC. Still, scatterplot diagrams suggested strong linear associations between the variables (Fig 4.6).

Table 4.3: A comparative table showing the overall results obtained:

Clinical Condition	**Study Site**				
	Wolgan Valley	Rylstone	Merriwa	Cessnock	Coolagolite
Mean Sarcoptic mange prevalence (%)	63.68	8.37	16.75	11.17	0
Mean Stress Level (FCM ng/g of dry weight)	53.75 ± 7.78 S.E.	11.88 ± 12.55 S.E.	62.52 ± 22.05 S.E.	35.98 ± 11.17 S.E.	12.75 ± 3.56 S.E.
Mean Strongyle egg count (e.p.g)	389.23 ± 144.30 S.E.	51.11 ± 19.75 S.E.	0.71 ± 1.04 S.E.	200 ± 200 S.E.	117.5 ± 86.14 S.E.

Our results confirmed that, of the five study sites, the Wolgan Valley site had the highest prevalence of bare-nosed wombats with sarcoptic mange. The Wolgan Valley also had the highest incidence of strongyle infection in the bare-nosed wombat population (Table 4.3). Balestrieri et al., (2006) have previously reported a positive correlation between the incidence of sarcoptic mange and parasitic helminth infection in foxes. Mangy foxes showed a higher prevalence of both cestodes and nematodes compared to foxes without sarcoptic mange. Furthermore, Balestrieri et al., (2006) suggested that in foxes as a consequence of sarcoptic

mange and helminth infection the host's protein intake is affected, thereby rendering the host undernourished and vulnerable to other infections. Indeed, the co-occurrence of the diseases could increase the host's susceptibility to sarcoptic mange, thus increasing disease progression and decreasing host life span. Similar observations were made in willow ptarmigan (*Lagopus lagopus*) where an increase in endoparasite load simultaneously increased the ectoparasite load in the birds (Holmstad et al., 2008).

A high incidence of sarcoptic mange and a high strongyle egg count in the bare-nosed wombats at the Wolgan Valley may be a result of habitat degradation as suggested previously by Hermsen, (2015) and Old et al., (unpublished). In another study, Martin et al., (1998) suggested habitat degradation as one of the possible reasons behind the widespread incidence of sarcoptic mange across the species range of bare-nosed wombats. Similar observations have been made in southern-hairy nosed wombats (*Lasiorhinus latifrons*) in the South Australian Murraylands, where habitat degradation has led to poor body condition of the wombats with occurrences of parasitic and bacterial diseases (Woolford et al., 2018). While common brushtail possums (*Trichosurus vulpecula*) were found resilient to anthropogenic changes in their habitat (Flynn, 2011), habitat degradation and fragmented landscape were reported to impact wild populations of antechinus (*Antechinus agilis*) (Johnstone et al., 2012) and squirrel glider (*Petaurus norfolcensis*) (Brearley et al., 2012).

Habitat degradation can lead to an alteration in food availability. A change in food availability can cause a change in infection pattern as has been witnessed in red colobus (*Piliocolobus tephrosceles*) (Chapman et al., 2006) and Panamanian howler monkeys *(Alouatta palliata)* (Milton, 1996). A variation of food availability and infection pattern can act as significant stressors for bare-nosed wombats, which may be the cause of the moderately high levels of FCM in the wombats at the Wolgan Valley (Table 4.3).

Few wombats with heavy sarcoptic mange infestation were observed foraging during day in the Wolgan Valley. This observation is similar to Hartley and English (2005), Simpson et al. (2016), Skerratt et al. (1999). Obligate exposure to an altered photoperiod and the increased vulnerability to predators can stimulate the HPA axis and secrete more stress hormones in these bare-nosed wombats.

Of the five study sites, the bare-nosed wombats at the Merriwa site were found to have the lowest strongyle egg counts. In contrast, these wombats had a moderately high level of sarcoptic mange incidence and the highest level of FCM (Table 4.3). Stress can increase the chances of disease susceptibility (Sapolsky, 2002). Skerratt, (1998) and Zumpt and Ledger, (1973) indicated that stress and underlying disease conditions may make wildlife prone to severe sarcoptic mange. Rescued wild koalas with high stress levels have been reported to have a high incidence of Chlamydia disease (Narayan and Vanderneut, 2019). Stressors such as vehicle collisions, bushfire, diseases and other injuries, and human interactions were reported to cause high physiological stress levels in these marsupials. Factors predisposing bare-nosed wombats to sarcoptic mange need to be assessed before planning conservation measures. Few examples of these factors or stressors can be observed in the captive wombat population at Cessnock, which are discussed below.

Cessnock is unique when compared to the other sites as the identity of the wombats from which the faecal samples were collected are known, as well as their sarcoptic mange incidence. Health information provided by the personnel at this site indicates that some wombats had endured attacks from other wombats, while one other wombat suffered from severe vitamin deficiency (Table 4.1). Besides, these wombats have been rescued from the wild after experiencing a stressful event such as vehicle collisions. A moderately high level of stress in these wombats

is likely due to the above-mentioned factors. The wombats at the Cessnock site had the second highest prevalence of sarcoptic mange and a moderately high level of endoparasitic burden (see Table 4.3), which may suggest an increase in disease susceptibility as a result of moderately high stress levels in this wombat population.

In comparison to other study sites, the bare-nosed wombats of Rylstone and Coolagolite had the lowest prevalence of sarcoptic mange and had the lowest FCM levels. On the other hand, the strongyle egg count in Coolagolite was much higher than the Rylstone site as shown in Table 4.3. The low sarcoptic mange prevalence and low FCM level may indicate a decrease in stress level in these two wombat populations, and hence a decreased sarcoptic mange prevalence. A higher level of FEC with lower stress levels in bare-nosed wombats at Coolagolite may suggest a higher tolerance to gut parasites in these wombats compared to other sites. Similar observations were made in captive female reindeers (*Rangifer tarandus tarandus*) (Carlsson et al., 2016) and in free-ranging racoons (*Procyon lotor*) (Monello et al., 2010), where no change in cortisol levels was reported between infected and uninfected animals, suggesting a tolerance of parasites within them.

Overall a low mean FEC has been observed for all study sites when compared to Gerhardt, (1996), who studied northern hairy-nosed wombats (*Lasiorhinus krefftii*). However, Gerhardt, (1996) suggested there were many limitations to the estimation of an endoparasitic burden when using the McMaster technique, such as the month of sample collection, age and species of the helminth laying eggs. Additionally, the host species should be taken into consideration since the tolerance to gut parasites can differ from species to species (Kutzer and Armitage, 2016). Consequently, it would be ideal to determine whether the interaction of these factors play a role in the endoparasitic burden of bare-nosed wombats. Moreover, as recommended by Gerhardt, (1996) conducting consistent sampling for a month would determine whether a

random sample collected could be used as an indicator of the endoparasitic burden for each of the five sites used in this study.

Results for the FEC at Merriwa should be interpreted with caution since there was a bias in faecal sample collection due to the time lag between collection of faecal samples and performing FEC analysis. A low FEC may have resulted from a longer storage period at 4°C but had mixed developmental stages of hatched larvae and unhatched eggs. Few adult non-viable larvae were observed during analysis (Fig 4.4 B), which would suggest that ideal aerobic conditions for egg hatching were met during the storage time and consequently led to a decrease in egg counts. Therefore, we recommend species-specific assessment of storage conditions prior to future FEC analysis or performing a molecular analysis of fresh faecal samples to enumerate the number of helminths.

The authors would like to emphasise that the results of our study were based on data collected from unidentified bare-nosed wombats from four free-ranging populations. Consequently, the authors do not have any data on the age, sex, pouch status, diet and health condition of the wombats from the Wolgan Valley, Rylstone, Merriwa and Coolagolite, since host-parasite interactions are influenced by these factors (Minchella and Scott, 1991). Additionally, results might differ with changes in humidity and precipitation rates. Besides, with the change of seasons, the availability of feed for the wombats also changes (Skerratt, 2001), which may impact the disease condition of the animals. Further studies should assess additional parameters such as age, sex, pouch status, health condition as well as seasonal factors to further determine the effect of stress and helminth loads in sarcoptic mange incidence in bare-nosed wombats.

4.6. Conclusion

The outcomes of this study suggest that chronic stress can be a significant factor which can increase the vulnerability to sarcoptic mange in free-living bare-nosed wombats. Martin et al., (1998) found an increase in susceptibility to sarcoptic mange in wombats during winter or drought seasons when the availability of feed is lower than in other months. Likewise, in a study on an American marsupial (*Gracilinanus agilis*) environmental stressors, such as the dry season, have been reported to increase disease susceptibility (Hernandez et al., 2018). In western gray squirrels (*Sciurus griseus*), prolonged habitat degradation along with mild winters as a result of climate change have been suggested to increase the frequency and severity of notoedric mange epizootics (Vander Haegen et al., 2018). Hence, malnutrition, habitat degradation and change of infection pattern can all act as potential chronic stressors of bare-nosed wombats. Over and above, chronic stress reduces immunocompetence and reduced immunocompetence increases the chances of infection (Cohen et al., 2012). Ecologists should consider chronic stress as an important risk factor for sarcoptic mange incidence in bare-nosed wombats when designing future conservation measures. In fact, high prevalence of sarcoptic mange can affect reproductive capacity as previously reported by Skerratt et al., (1999) in mature wombats infested with sarcoptic mange, where the gonads were not active or minimally active. Sarcoptic mange can consequently reduce the abundance of bare-nosed wombats (Martin et al., 1998), which, in addition to reduced fecundity can induce extinction in local bare-nosed wombat populations.

Conflict of Interest

The authors declare no conflict of interest.

4.7 Acknowledgements

The authors are grateful to Wolgan Valley, Mike and Sue Pridmore of Badger Ground Wildlife Sanctuary, Rylstone, Ted and Jenny Finnie of Eagle's Drift, Merriwa, Daniel Ondinea of Coolagolite and to Roz and Kevin Holmes of Cedar Creek Wombat Rescue Inc. and Hospital for giving permission to collect bare-nosed wombat faecal samples and conducting spotlighting surveys on their properties. The authors thank Rowan Thorley, Blaire Vallin Brooke A. Crismale, Emily Gallagher and Tyler Philpott who volunteered during spotlighting surveys. Mark Emanuel provided technical assistance. This project is supported by research funds allocated by WSU SSH to CS for her MPhil research and donations provided to Wombat Giving (JO). Procedures were approved by the Western Sydney University Animal Care and Ethics Committee (Protocol number: A12033), Western Sydney University Biosafety and Radiation Safety Committee (Protocol number: B10524) and NSW National Parks and Wildlife Services (Licence number: S13202).

4.8 References

Alasaad, S., Walton, S., Rossi, L., Bornstein, S., Abu-Madi, M., Soriguer, R.C., et al., 2011. Sarcoptes-world molecular network (Sarcoptes-WMN): integrating research on scabies. Int. J. Infect. Dis. 15 e294-e297. https://doi.org/10.1016/j.ijid.2011.01.012.

Arlian, L.G., 1989. Biology, host relations, and epidemiology of *Sarcoptes scabiei*. Annu. Rev. Entomol. 34 139-159. https://doi.org/10.1146/annurev.en.34.010189.001035.

Astorga, F., Carver, S., Almberg, E.S., Sousa, G.R., Wingfield, K., Niedringhaus, K.D., et al., 2018. International meeting on sarcoptic mange in wildlife, June 2018, Blacksburg, Virginia, USA. Parasit Vectors. 11 449-449. 10.1186/s13071-018-3015-1.

Balestrieri, A., Remonti, L., Ferrari, N., Ferrari, A., Valvo, T.L., Robetto, S., et al., 2006. Sarcoptic mange in wild carnivores and its co-occurrence with parasitic helminths in the Western Italian Alps. Eur. J. Wildl. Res. 52 196-201. https://doi.org/10.1007/s10344-006-0036-4.

Brearley, G., McAlpine, C., Bell, S., Bradley, A., 2012. Influence of urban edges on stress in an arboreal mammal: a case study of squirrel gliders in southeast Queensland, Australia. Landsc. Ecol. 27 1407-1419.

Canfield, P.J., Love, D.N., Mearns, G., Farram, E., 1991. Chlamydial infection in a colony of captive koalas. Aust. Vet. J. 68 167-169. 10.1111/j.1751-0813.1991.tb03171.x.

Carlsson, A.M., Mastromonaco, G., Vandervalk, E., Kutz, S., 2016. Parasites, stress and reindeer: infection with abomasal nematodes is not associated with elevated glucocorticoid levels in hair or faeces. Conserv Physiol. 4 58. 10.1093/conphys/cow058.

Chapman, C.A., Wasserman, M.D., Gillespie, T.R., Speirs, M.L., Lawes, M.J., Saj, T.L., et al., 2006. Do food availability, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? Am. J. Phys. Anthropol. 131 525-534. https://doi.org/10.1002/ajpa.20477.

Cizauskas, C.A., Turner, W.C., Pitts, N., Getz, W.M., 2015. Seasonal Patterns of Hormones, Macroparasites, and Microparasites in Wild African Ungulates: The Interplay among Stress, Reproduction, and Disease. PLoS One. 10. 10.1371/journal.pone.0120800.

Cohen, S., Janicki-Deverts, D., Doyle, W.J., Miller, G.E., Frank, E., Rabin, B.S., et al., Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. In: McEwen, B.S., (Ed.), Proceedings of the National Academy of Sciences, U.S.A, 2012, pp. 5995-5999.

Corner, L.A., Bagust, T.J., 1993. Australian standard diagnostic techniques for animal diseases. CSIRO for the Australian Agricultural Council (Standing Committee on Agriculture) East Melbourne, Vic., CSIRO.

Dantzer, B., Fletcher, Q.E., Boonstra, R., Sheriff, M.J., 2014. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? Conserv Physiol. 2. 10.1093/conphys/cou023.

Evans, M., 2008. Home range, burrow-use and activity patterns in common wombats (*Vombatus ursinus*). Wildl. Res. 35 455-462. https://doi.org/10.1071/WR07067.

Flynn, E.M., Exploring the influence of disturbance history and forest type on an Arboreal Marsupial, the Common Brushtail Possum (Trichosurus Vulpecula), using a Multi-disciplinary Approach: University of Tasmania; 2011.

Fraser, T.A., Charleston, M., Martin, A., Polkinghorne, A., Carver, S., 2016. The emergence of sarcoptic mange in Australian wildlife: an unresolved debate. Parasit Vectors. 9 316. 10.1186/s13071-016-1578-2.

Gerhardt, K., The Parasite Load of the Northern Hairy-Nosed Wombat *Lasiorhinus Krefftii* (Owen, 1872): Central Queensland University; 1996.

Goldstein, E.J., Millspaugh, J.J., Washburn, B.E., Brundige, G.C., Raedeke, K.J., 2005. Relationships among fecal lungworm loads, fecal glucocorticoid metabolites, and lamb recruitment in free-ranging Rocky Mountain bighorn sheep. J. Wildl. Dis. 41 416-425. https://doi.org/10.7589/0090-3558-41.2.416.

Gulland, F., 1992. The role of nematode parasites in Soay sheep (Ovis aries L.) mortality during a population crash. Parasitology. 105 493-503.

Hartley, M., English, A., 2005. *Sarcoptes scabei var. wombati* infection in the common wombat (*Vombatus ursinus*). Eur. J. Wildl. Res. 51 117-121. https://doi.org/10.1007/s10344-005-0080-5.

Hermsen, E., Analysis of non-invasive sampling techniques to determine sarcoptic mange distribution and MHC diversity in the bare-nosed wombat (*Vombatus ursinus*): Western Sydney University; 2015.

Hernandez, S., Strona, A., Leiner, N., Suzán, G., Romano, M., 2018. Seasonal changes of faecal cortisol metabolite levels in *Gracilinanus agilis* (Didelphimorphia: Didelphidae) and its association to life histories variables and parasite loads. Conserv Physiol. 6 coy21. https://doi.org/10.1093/conphys/coy021.

Hing, S., Narayan, E., Thompson, R.A., Godfrey, S., 2014. A review of factors influencing the stress response in Australian marsupials. Conserv Physiol. 2 cou027. https://doi.org/10.1093/conphys/cou027.

Holmstad, P.R., Jensen, K.H., Skorping, A., 2008. Ectoparasite intensities are correlated with endoparasite infection loads in willow ptarmigan. Oikos. 117 515-520. https://doi.org/10.1111/j.0030-1299.2008.16219.x.

Johnstone, C.P., Lill, A., Reina, R.D., 2012. Does habitat fragmentation cause stress in the agile antechinus? A haematological approach. J. Comp. Physiol. B. 182 139-155.

Kindermann, C., Narayan, E.J., Hero, J.-M., 2017. Does physiological response to disease incur cost to reproductive ecology in a sexually dichromatic amphibian species? Comp. Biochem. Physiol., Part A Mol. Integr. Physiol. 203 220-226. https://doi.org/10.1016/j.cbpa.2016.09.019.

Kutzer, M.A., Armitage, S.A., 2016. Maximising fitness in the face of parasites: a review of host tolerance. Zoology. 119 281-289. https://doi.org/10.1016/j.zool.2016.05.011.

Lello, J., Boag, B., Hudson, P., 2005. The effect of single and concomitant pathogen infections on condition and fecundity of the wild rabbit (Oryctolagus cuniculus). International journal for parasitology. 35 1509-1515.

Martin, A.M., Burridge, C.P., Ingram, J., Fraser, T.A., Carver, S., 2018. Invasive pathogen drives host population collapse: Effects of a travelling wave of sarcoptic mange on bare-nosed wombats. J. Appl. Ecol. 55 331-341. https://doi.org/10.1111/1365-2664.12968.

Martin, R., Handasyde, K., Skerratt, L., 1998. Current distribution of sarcoptic mange in wombats. Aust. Vet. J. 76 411-414. https://doi.org/10.1111/j.1751-0813.1998.tb12391.x.

Milton, K., 1996. Effects of bot fly (*Alouattamyia baeri*) parasitism on a free-ranging howler monkey (*Alouatta palliata*) population in Panama. J. Zool. 239 39-63. https://doi.org/10.1111/j.1469-7998.1996.tb05435.x.

Minchella, D.J., Scott, M.E., 1991. Parasitism: a cryptic determinant of animal community structure. Trends Ecol. Evol. 6 250-254. https://doi.org/10.1016/0169-5347(91)90071-5.

Monello, R., Millspaugh, J., Woods, R., Gompper, M., 2010. The influence of parasites on faecal glucocorticoid metabolite levels in raccoons: an experimental assessment in a natural setting. J. Zool. 282 100-108. https://doi.org/10.1111/j.1469-7998.2010.00717.x.

Narayan, E., Vanderneut, T., 2019. Physiological Stress in Rescued Wild Koalas Are Influenced by Habitat Demographics, Environmental Stressors, and Clinical Intervention. Front. Endocrinol. (Lausanne). 10. 10.3389/fendo.2019.00018.

Old, J.M., Hunter, N., Wolfenden, J., The preferred habitat, burrow characteristics and levels of mange in a population of bare-nosed wombats (*Vombatus ursinus*). unpublished.

Old, J.M., Sengupta, C., Narayan, E., Wolfenden, J., 2017. Sarcoptic mange in wombats—A review and future research directions. Transbound. Emerg. Dis. 65 399-407. 10.1111/tbed.12770.

Pence, D.B., Ueckermann, E., 2002. Sarcoptic mange in wildlife. Rev. Sci. Tech. 21 385-398.

Roger, E., Laffan, S.W., Ramp, D., 2011. Road impacts a tipping point for wildlife populations in threatened landscapes. Popul. Ecol. 53 215-227. https://doi.org/10.1007/s10144-010-0209-6.

Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. Trends Ecol. Evol. 19 249-255. https://doi.org/10.1016/j.tree.2004.03.008.

Sapolsky, R.M., 2002. Endocrinology of the stress-response. In: Becker, J.B., Breedlove, D., (Eds.), Behavioral endocrinology, 2nd ed.

Schwarzenberger, F., 2007. The many uses of non-invasive faecal steroid monitoring in zoo and wildlife species. Int Zoo Yearb. 41 52-74. https://doi.org/10.1111/j.1748-1090.2007.00017.x.

Sengupta, C., Old, J.M., Narayan, E., Under Review. Testing the environmental decay of faecal cortisol metabolites in bare-nosed wombats (*Vombatus ursinus*) Gen. Comp. Endocrinol.

Sheriff, M.J., Dantzer, B., Delehanty, B., Palme, R., Boonstra, R., 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia. 166 869-887. https://doi.org/10.1007/s00442-011-1943-y.

Skerratt, L.F., 1998. Diseases and parasites of the common wombat *Vombatus ursinus* in the Healesville area of Victoria. In: Wells, R., Pridmore, P., (Eds.), Wombats, Surrey Beatty & Sons, Chipping, Norton.

Skerratt, L.F., Sarcoptic mange in the common wombat, *Vombatus ursinus* (Shaw, 1800): Univeristy of Melbourne; 2001.

Skerratt, L.F., 2003. Cellular response in the dermis of common wombats (*Vombatus ursinus*) infected with *Sarcoptes scabiei var. wombati*. J. Wildl. Dis. 39 193-202. https://doi.org/10.7589/0090-3558-39.1.193.

Skerratt, L.F., Middleton, D., Beveridge, I., 1999. Distribution of life cycle stages of Sarcoptes scabiei var wombati and effects of severe mange on common wombats in Victoria. J. Wildl. Dis. 35 633-646. https://doi.org/10.7589/0090-3558-35.4.633.

Snieszko, S., 1974. The effects of environmental stress on outbreaks of infectious diseases of fishes. J. Fish Biol. 6 197-208.

Thompson, R.C.A., Lymbery, A.J., Smith, A., 2010. Parasites, emerging disease and wildlife conservation. Int. J. Parasitol. 40 1163-1170. http://doi.org/10.1016/j.ijpara.2010.04.009.

Tompkins, D.M., Carver, S., Jones, M.E., Krkošek, M., Skerratt, L.F., 2015. Emerging infectious diseases of wildlife: a critical perspective. Trends Parasitol. 31 149-159. https://doi.org/10.1016/j.pt.2015.01.007.

Touma, C., Palme, R., 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Ann. N. Y. Acad. Sci. 1046 54-74. https://doi.org/10.1196/annals.1343.006.

Triggs, B., 2009. Wombats, 2nd ed. CSIRO PUBLISHING Collingwood, Vic.

Vander Haegen, W.M., Orth, G.R., Johnston, A.N., Linders, M.J., 2018. Endemic diseases affect population dynamics of tree squirrels in contrasting landscapes. J. Wildl. Manage. 82 328-343. https://doi.org/10.1002/jwmg.21383.

Whitlock, H., 1948. Some modifications of the McMaster helminth egg-counting technique and apparatus. Journal of the Council for Scientific and Industrial Research. Australia. 21 177-180.

Wikelski, M., Cooke, S.J., 2006. Conservation physiology. Trends Ecol. Evol. 21 38-46. https://doi.org/10.1016/j.tree.2005.10.018.

Wolfenden, J., Old, J.M., Taking Action to Improve Wombat Health on Emirates Estates Wolgan Conservation Area: Final Report 2012. University of Western Sydney, Native and Pest Animal Unit, Richmond NSW, 2012.

Woolford, L., Boardman, W., Caraguel, C., Taggart, D., Lethbridge, M., Southern hairy nosed wombats of the Murraylands: a survey of health and disease 2011-2016. Wombats through time and space, Adelaide, Australia, 2018.

Zumpt, F., Ledger, J., 1973. Present epidemiological problems of sarcoptic mange in wild and domestic animal. S. Afr. J. Wildl. Res. 3 119-120.

Hartley, M. & English, A. 2005. *Sarcoptes scabei var. wombat*i infection in the common wombat (*Vombatus ursinus*). *European Journal of Wildlife Research,* **51,** 117-121

Simpson, K., Johnson, C. N. & Carver, S. 2016. Sarcoptes scabiei: the mange mite with mighty effects on the common wombat (*Vombatus ursinus*). *PloS one,* **11,** e0149749

Skerratt, L. F., Middleton, D. & Beveridge, I. 1999. Distribution of life cycle stages of *Sarcoptes scabiei var wombati* and effects of severe mange on common wombats in Victoria. *Journal of Wildlife Diseases,* **35,** 633-646

Chapter 5: General discussion, recommendations and future directions

5.1 General discussion

Sarcoptic mange, its effects on bare-nosed wombats and current treatment regime have been reviewed in detail (Chapter 2). Optimisation experiments on bare-nosed wombat faecal samples demonstrated baseline FCM levels and the decay rate of FCMs in autumn (Chapter 3). Furthermore, in this thesis, I demonstrated two EIAs that can be used to validate the adrenocortical activity of bare-nosed wombats (Chapter 3). Population density, sarcoptic mange prevalence and endoparasitic burden in bare-nosed wombat populations at five locations in N.S.W Australia were determined (Chapter 4). My research was also the first to investigate the role of chronic stress and endoparasitic burden in the incidence of sarcoptic mange in bare-nosed wombats (Chapter 4). All experiments conducted in this study were non-invasive.

Non-invasive techniques do not require capture and restraint of the animals which can often lead to undue stress, furthermore, non-invasive techniques are much simpler and more cost-effective than invasive methods (Whitten et al., 1998). In this study, spotlighting was conducted to determine the bare-nosed wombat densities and to estimate sarcoptic mange prevalence in the wombats. Although an indirect technique to determine population density, spotlighting is less laborious and does not cause unnecessary stress to the wombats as can occur during capture-mark-recapture (McIlroy, 1977), and is much less expensive compared to DNA technologies to identify individuals (Banks et al., 2002). Furthermore, the present study employed non-invasive faecal sampling to determine the physiological stress level and the endoparasitic burden of bare-nosed wombats. Endoparasitic burden can also be determined by surgically removing the gut contents of the wombats (Skerratt, 1998) or by retrieving the faecal samples directly from the rectum (Zajac and Conboy, 2012), however conducting such

procedures not only cause distress to the animal but can be detrimental. Utilising faeces, urine, feather, hair and blow air (from whales) to monitor the HPA-axis activity of an animal have been gaining popularity over the past few decades (Burgess et al., 2016, Dantzer et al., 2014, Palme, 2019, Sheriff et al., 2011). Stress hormone metabolites (GC metabolites) in faeces reflect not only the true baseline GC level but also indicate the integrated average of plasma-free GCs that have been metabolized and excreted throughout a species-specific time period (Goymann, 2009, Palme et al., 2005).

5.1.1 Sarcoptic mange in wombats – a review and future directions

The primary aim of this thesis was to explore the reasons for increased vulnerability to sarcoptic mange in bare-nosed wombats. The literature review (Chapter 2) identified gaps in the literature.

This chapter gives a comprehensive review on sarcoptic mange and its worldwide epidemics, in addition to a detailed description of the mite (*Sarcoptes scabiei*), and the infection caused by the mite. The review provides a thorough knowledge on the origin of the disease and its host-specificity. The damaging effects of the disease on bare-nosed wombats has been detailed while the treatment regime that is currently practised has been critically reviewed.

The review reports that current treatment practices are successful for captive wombat populations however, free-ranging wombat populations have a higher chance of reinfection if treated when there are high densities and due to their burrow sharing behaviour. The current treatment regime is therefore not practical for all free-ranging populations. Vaccinations may be one solution, however, the knowledge and selection of suitable antigens to design the vaccine are still limited. Consequently, to conserve the free-ranging populations of wombats from sarcoptic mange, controlling the parameters (such as stress due to anthropogenic

disturbances and secondary endoparasitic infections) that increase the vulnerability of these marsupials to the disease would be a more desirable solution.

Apart from providing a broad overview into sarcoptic mange and its effects on bare-nosed wombats this review also reports on other threats to the survival of wombats, which include anthropogenic disturbance, habitat degradation, vehicle collisions, climate change and other diseases such as toxoplasmosis and gut parasites. These threats or chronic stressors may be the reason for the increased prevalence of sarcoptic mange in bare-nosed wombats. Therefore, the objective of this thesis was to investigate if chronic stress and endoparasitic burden play a role in sarcoptic mange incidence in bare-nosed wombats.

5.1.2 Testing the environmental decay of faecal cortisol metabolites in bare-nosed wombats (Vombatus ursinus)

An optimisation study was conducted initially to determine the baseline stress hormone metabolite level in faecal samples of captive bare-nosed wombats. This study further determined the decay rate of FCMs, since faecal samples obtained in the wild are usually < 12h old. In addition, this study further validated two EIA techniques to successfully quantify the FCMs in the scat samples of the wombats.

Baseline FCM level in captive bare-nosed wombats ranges between 23.95 - 8.55 ng/g of dry faecal weight (Sengupta et al., Under Review). The mean FCM level remained stable for 120h in samples collected during autumn. Validation tests disclosed that an in-house cortisol EIA (R4866; C. Munro, UC Davis, USA; 1:15000 working dilution) and a commercially available cortisol EIA kit (Detect X ® Cortisol Enzyme Immuno Assay Ann Arbor ®, MI, USA) can be successfully used to quantify FCM levels in captive bare-nosed wombats. For comparative knowledge on the decay rate of other animals see Table 3.1.

5.1.3 Evaluating the role of stress and helminth load in sarcoptic mange incidence in bare-nosed wombats (Vombatus ursinus) in N.S.W., Australia.

Except for surveys conducted by Hermsen (2015) and Hunter (2011), two decades have passed since the last survey of sarcoptic mange prevalence in bare-nosed wombat populations were conducted by Martin et al. (1998). Determining the current prevalence of sarcoptic mange and population density estimates of bare-nosed wombats were therefore necessary, however, it was beyond the scope of this thesis to investigate all bare-nosed populations. Consequently, five representative populations located in N.S.W. were chosen (Chapter 4).

The number of spotlighting surveys conducted on wombats in this study is comparable to the surveys done in studies by Wells (1978), McIlroy (1977), Hunter (2011) and Hermsen (2015). The mean wombat densities observed by conducting spotlighting survey in Wolgan Valley, Rylstone, Merriwa and Coolagolite were 1.55 ha^{-1}, 0.20 ha^{-1}, 0.07 ha^{-1} and 0.02 ha^{-1} respectively. The wombat density at the Wolgan Valley has increased since Hermsen (2015) (0.20 ha^{-1}) but have decreased since Hunter (2011) (11.5 ha^{-1}). Similarly, population densities of wombats at Rylstone and Merriwa have decreased when compared to the data recorded by Hermsen (2015) (0.34 ha^{-1} and 0.17 ha^{-1} respectively). On comparing with previous researchers, it is likely that the overall wombat density along the transect at Wolgan Valley observed per night (42.42 ± 4.52 S.E) have increased slightly [Hermsen (2015) (39 ± 4.24 S.E) and Hunter (2011) (29.5)] while, a decrease in the overall mean number of wombats observed per night was reported at Rylstone (9 ± 2) and Merriwa (6 ± 0) [Hermsen (2015) (30 ± 6.44 S.E and 13.8 ± 1.07 S.E respectively)]. Wombat density on a given night is influenced by food availability, presence of water, sites for burrow construction, temperature, breeding season, overlap of home ranges, breeding season, wind and moon phases as well as the season (Borchard, 2013, Borchard et al., 2008, Hermsen, 2015, Skerratt et al., 2004). It has been reported that wombat density increases during the wet season of July/August (Hermsen, 2015), which is indeed true

since the wombat spotlighting survey in Rylstone and Merriwa was conducted during the drier months of February, May and June whereas most spotlighting surveys conducted in the Wolgan Valley were during July and August.

Sarcoptic mange was observed in three of the four sites where spotlighting was conducted, with a mean sarcoptic mange prevalence of 63.68%, 8.37% and 16.75% obtained at Wolgan Valley, Rylstone and Merriwa respectively. In Coolagolite no wombats showing clinical signs of sarcoptic mange were observed, and in Cessnock, the sarcoptic mean mange prevalence of the captive bare-nosed wombat population was 11.17%. Sarcoptic mange prevalence in the current study is comparable to Martin et al. (1998), who reported sarcoptic mange prevalence in bare-nosed wombats to be 21.7%, 0%, 22.2% and 15% in three localities in Victoria and one locality in N.S.W respectively, whereas Skerratt (1998), found severe chronic sarcoptic mange in 11% of bare-nosed wombats in Healesville, Victoria. Furthermore, sarcoptic mange prevalence reported by Ruykys et al. (2009) was found to be 76% in southern hairy-nosed wombats in Murraylands, South Australia during February to July 2005. Recent survey records in 2017 reported sarcoptic mange prevalence in Tasmanian bare-nosed wombats ranges between 0 – 6% (https://dpipwe.tas.gov.au/, 2018). Sarcoptic mange prevalence in Merriwa is similar to the data (16%) reported by Hermsen (2015) whereas sarcoptic mange prevalence in Rylstone decreased when compared to Hermsen (2015) (16%). When compared to the current study, sarcoptic mange prevalence in the Wolgan Valley increased significantly since Hermsen (2015) (24%) and by Hunter (2011) (20%). Parameters such as wombat density, food availability, endoparasitic burden and environmental stress factors (Martin et al., 1998, Old et al., unpublished, Skerratt et al., 1998) can influence sarcoptic mange prevalence in bare-nosed wombats. A high wombat density in the Wolgan Valley can correspond to the high sarcoptic mange prevalence in these marsupials. In the current study, the role of endoparasitic burden and chronic stress in sarcoptic mange incidence in bare-nosed wombats was investigated.

In this study, the endoparasitic burden at Wolgan Valley, Rylstone, Merriwa, Coolagolite and Cessnock were reported (Table 4.3 in Chapter 4). Although the overall egg count was low when compared to Gerhardt (1996) and Hunter (2011) a strong association was observed between mean sarcoptic mange prevalence and mean faecal egg count. A high incidence of sarcoptic mange and a high strongyle egg count in the bare-nosed wombats at the Wolgan Valley was observed in the current study as well as by Old et al. (unpublished). A similar association between ectoparasitic and endoparasitic load was made in willow ptarmigan (*Lagopus lagopus*) (Holmstad et al., 2008) and red foxes in the Italian Alps (Balestrieri et al., 2006). Balestrieri et al. (2006) reported that the host suffers a poor protein intake as a consequence of multiple infections thereby rendering the host undernourished and more prone to other infections.
They further reported that coinfection with multiple parasites could exacerbate the effects of sarcoptic mange. In merino sheep, the intestinal worm burden was reported to influence the external louse burden which may be due to immunosuppressive effects of ectoparasite and endoparasite load in addition to poor host nutrition and stress (James et al., 2002).

The current study reported the FCM level at the Wolgan Valley, Rylstone, Merriwa, Coolagolite and Cessnock (Table 4.3). Three of the five study sites had a comparatively higher FCM level than the baseline level (23.95 - 8.55 ng/g of dry faecal weight) reported in captive bare-nosed wombats. A strong positive correlation was observed between sarcoptic mange prevalence and chronic stress in the five study sites in N.S.W. Similar observation were reported in an American marsupial (*Gracilinanus agilis)* where chronic stressors (dry season) were related to increasing disease vulnerability (Hernandez et al., 2018). In western gray squirrels (*Sciurus griseus*) prolonged habitat degradation along with mild winters as a result of climate change have been suggested to increase the frequency and severity of notoedric mange epizootics (Vander Haegen et al., 2018).

Habitat degradation has previously been reported in the Wolgan Valley, Rylstone and Merriwa (Hermsen, 2015, Old et al., unpublished). Habitat degradation leads to a change in food availability as well as a change of infection pattern (Chapman et al., 2006). High animal density has also been related to increased vulnerability to ectoparasites in colonial cliff swallows (*Petrochelidon pyrrhonota*), in southwestern Nebraska, USA (Raouf et al., 2006). Martin et al. (1998) found an increase in susceptibility to sarcoptic mange in bare-nosed wombats during winter or during drought when the availability of feed is lower than in other months. Therefore, parameters such as food scarcity, habitat degradation, change of infection pattern, and wombat density increases are potential risk factors which can aggravate the chances of infection to sarcoptic mange in bare-nosed wombats. Chronic stressors can compromise immunocompetence which can exacerbate the vulnerability to other infections. Thus, chronic stress can affect sarcoptic mange susceptibility in free-ranging bare-nosed wombats. Future researchers are therefore encouraged to design conservation studies which minimise chronic stressors to minimise the prevalence of sarcoptic mange.

5.2 Recommendations and future directions

As indicated in Chapter 3, FCMs levels can be influenced by many factors including sex of the subject animals, their diet, the ambient temperature, intestinal bacterial composition (Goymann, 2012) their seasonal and daily rhythm, body condition and sample mass, sample age and preservation conditions (Khan et al., 2002, Millspaugh and Washburn, 2004). These factors can not only affect the type of hormone metabolite formed but can also affect the decay rate of the FCMs. Therefore, it is recommended to conduct biological validation tests such as ACTH challenge in addition to biochemical validations. Furthermore, future research should

test other commercially available EIAs to determine the most robust EIA for testing non-invasive steroid profiles in wombats.

The pilot study (Chapter 3) was conducted in autumn in NSW, Australia, when the precipitation and humidity rates are different from winter and summer months. Therefore, although our study confirms collecting wild bare-nosed wombat faecal samples within 12 h -120 h after deposition will ensure reliable FCM EIA results, collection of fresh faecal samples is recommended since the weather variables are likely to differ in other seasons.

Although the overall wombat density (Chapter 4) at the Wolgan Valley was higher than Hermsen (2015) the density at Rylstone and Merriwa was much lower. However, spotlighting is an indirect method that can be used to measure population density of wild wombats and that burrow activity details should also be taken into account and may provide a more accurate representation of the wombat population (Hermsen, 2015, McIlroy, 1977). Therefore, future researchers should consider including burrow count survey along with spotlighting.

The FCM, FEC and sarcoptic mange prevalence data from this study (Chapter 4) have indicated a lack of evidence of any causal relationship between FEC and mean sarcoptic prevalence although a strong positive correlation was observed between mean FCM and mean sarcoptic prevalence. This study does provide preliminary insight into the level of stress, endoparasitic burden and sarcoptic mange prevalence of the bare-nosed wombat population located in five different sites in NSW. Overall, free-ranging bare-nosed wombats in three (Wolgan Valley, Merriwa and Cessnock) of the five study sites had mean stress levels (Chapter 4) higher than the baseline stress level observed in captive wombats (Chapter 3). In addition, the mean sarcoptic mange prevalence at these three sites (Wolgan Valley, Merriwa and Cessnock) were higher among the remaining locations in NSW. This study suggests that chronic stress can be a factor in increasing vulnerability to sarcoptic mange in wild bare-nosed wombats. Therefore,

it is recommended that future ecologists and wildlife managers should consider the factors that predispose the bare-nosed wombats to sarcoptic mange before planning conservation measures. The overall endoparasitic burden in bare-nosed wombat populations at the five study sites was low (Chapter 4) when compared to previous studies (Gerhardt, 1996, Hunter, 2011). Estimation of an endoparasitic burden when using the McMaster technique has many limitations (Gerhardt, 1996), such as the month of sample collection, age and species of the helminth shedding eggs. In addition, the tolerance to gut parasites is species-specific therefore host species should be taken into account (Kutzer and Armitage, 2016). Consequently, it is likely that these factors play a role on the endoparasitic burden of bare-nosed wombats. Furthermore, the faecal samples were collected once during the study from the five sites, and therefore the FEC results may not be representative of the true endoparasitic burden of each population. Hence, as suggested by Gerhardt (1996), it is recommended that consistent sampling for a month be undertaken to determine whether random sample collection is representative of FEC levels.

As indicated in Chapter 4, readers should interpret the results of endoparasitic burden in Merriwa with caution since a considerable time gap was present between sample collection and conducting FEC analysis. A low FEC in the Merriwa wombat population may be the result of keeping the faecal samples for a prolonged period at 4°C as a consequence of which few adult non-viable larvae were observed during analysis. Therefore, it is recommended that future research should keep in mind that prolonged storage of bare-nosed wombat faecal samples at 4°C can lead to a decrease in faecal egg counts. Furthermore, a pilot study to reveal the ideal species-specific storage conditions or molecular analysis (such as qPCR) on fresh faecal samples may enumerate the number of helminths.

Host-parasite interactions, as mentioned in Chapter 4, are influenced by a number of parameters such as age, sex, reproductive status, diet and health condition of the host (Minchella and Scott,

1991). Information regarding these parameters was obtained for the captive wombat population of Cessnock, however the same was not possible for the wild wombat populations located in the Wolgan Valley, Rylstone, Merriwa and Coolagolite. Additionally, as mentioned in Chapter 3 and Chapter 4, seasonal change can result in variable FCM levels. A change of season likely also signifies a change of food availability for the wombats (Skerratt, 2001), which can impact disease condition. It would therefore be interesting to expand the parameters such as age, sex, reproductive status, and health condition of wombats, as well as seasonal effects on the sarcoptic mange status in the bare-nosed wombats. Future researchers should design their study, including the additional parameters, to gain further insights into the role of stress and endoparasitic burden in sarcoptic mange incidence in bare-nosed wombats. Future studies may investigate the use of hair and faecal samples to genetically identify individual wombats, as well as estimate population densities, as described previously in bare-nosed wombats, southern hairy-nosed wombats and the endangered northern hairy-nosed wombats (Banks et al., 2002, Sloane et al., 2000, Walker et al., 2006).

5.3 Conclusion

My thesis focused on exploring the role of endoparasitic burden, stress level and sarcoptic mange prevalence using non-invasive methods. This study is the first to validate two EIAs successfully for monitoring stress levels in captive bare-nosed wombats. The baseline stress level of bare-nosed wombats as well as the decay rate of FCMs in autumn were reported. This study is also the first to report the mean stress level, mean endoparasitic burden and mean sarcoptic mange prevalence of five bare-nosed wombat populations located in NSW, Australia. Although further investigations are necessary, this study suggested that chronic stress can increase the susceptibility of sarcoptic mange in wild free-ranging bare-nosed wombats.

5.4 References

Balestrieri, A., Remonti, L., Ferrari, N., Ferrari, A., Valvo, T. L., Robetto, S. & Orusa, R. 2006. Sarcoptic mange in wild carnivores and its co-occurrence with parasitic helminths in the Western Italian Alps. *European Journal of Wildlife Research,* **52,** 196-201

Banks, S. C., Piggott, M. P., Hansen, B. D., Robinson, N. A. & Taylor, A. C. 2002. Wombat coprogenetics: enumerating a common wombat population by microsatellite analysis of faecal DNA. *Australian Journal of Zoology,* **50,** 193-204 https://doi.org/10.1071/ZO01072

Borchard, P. 2013. Using camera trap data to model habitat use by wombats and cattle in Australian riparian ecosystems. *Applied Ecology and Environmental Research,* **11,** 21-33

Borchard, P., Mcilroy, J. & Mcarthur, C. 2008. Links between riparian characteristics and the abundance of common wombat (*Vombatus ursinus*) burrows in an agricultural landscape. *Wildlife Research,* **35,** 760-767

Burgess, E. A., Hunt, K. E., Kraus, S. D. & Rolland, R. M. 2016. Get the most out of blow hormones: validation of sampling materials, field storage and extraction techniques for whale respiratory vapour samples. *Conservation physiology,* **4** 10.1093/conphys/cow024

Chapman, C. A., Wasserman, M. D., Gillespie, T. R., Speirs, M. L., Lawes, M. J., Saj, T. L. & Ziegler, T. E. 2006. Do food availability, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? *American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists,* **131,** 525-534

Dantzer, B., Fletcher, Q. E., Boonstra, R. & Sheriff, M. J. 2014. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? *Conservation Physiology,* **2**

Gerhardt, K. 1996. The Parasite Load of the Northern Hairy-Nosed Wombat *Lasiorhinus krefftii* (Owen, 1872).

Goymann, W. 2009. Noninvasive monitoring of hormones in bird droppings: physiological validation, sampling, extraction, sex differences, and the influence of diet on hormone metabolite levels. *Annals of the New York Academy of Sciences,* **1046,** 35-53 https://doi.org/10.1196/annals.1343.005

Goymann, W. 2012. On the use of non-invasive hormone research in uncontrolled, natural environments: the problem with sex, diet, metabolic rate and the individual. *Methods in Ecology and Evolution,* **3,** 757-765

Hermsen, E. 2015. Analysis of non-invasive sampling techniques to determine sarcoptic mange distribution and MHC diversity in the bare-nosed wombat (*Vombatus ursinus*). Bachelor of Science, Western Sydney University.

Hernandez, S., Strona, A., Leiner, N., Suzán, G. & Romano, M. 2018. Seasonal changes of faecal cortisol metabolite levels in *Gracilinanus agilis* (Didelphimorphia: Didelphidae) and its association to life histories variables and parasite loads. *Conservation Physiology,* **6,** coy21 https://doi.org/10.1093/conphys/coy021

Holmstad, P. R., Jensen, K. H. & Skorping, A. 2008. Ectoparasite intensities are correlated with endoparasite infection loads in willow ptarmigan. *Oikos,* **117,** 515-520

Https://Dpipwe.Tas.Gov.Au/. 2018. [Accessed].

Hunter, N. 2011. The effect of sarcoptic mange on the ecology of the common wombat *(Vombatus ursinus)*. Bachelor of Science (Honours) thesis, University of Western Sydney.

James, P. J., Carmichael, I. H. C., Pfeffer, A., Martin, R. R. & O'callaghan, M. G. 2002. Variation among Merino sheep in susceptibilty to lice (*Bovicola ovis*) and association with susceptibility to trichostrongylid gastrointestinal parasites. *Veterinary Parasitology,* **103,** 355-365 https://doi.org/10.1016/S0304-4017(01)00601-X

Khan, M., Altmann, J., Isani, S. & Yu, J. 2002. A matter of time: evaluating the storage of fecal samples for steroid analysis. *General and comparative endocrinology,* **128,** 57-64

Kutzer, M. A. & Armitage, S. A. 2016. Maximising fitness in the face of parasites: a review of host tolerance. *Zoology,* **119,** 281-289

Martin, R., Handasyde, K. & Skerratt, L. 1998. Current distribution of sarcoptic mange in wombats. *Australian veterinary journal,* **76,** 411-414

Mcilroy, J. 1977. Aspects of the ecology of the common wombat, *Vombatus ursinus* II. Methods for estimating population numbers. *Wildlife Research,* **4,** 223-228

Millspaugh, J. J. & Washburn, B. E. 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. *General and comparative endocrinology,* **138,** 189-199

Minchella, D. J. & Scott, M. E. 1991. Parasitism: a cryptic determinant of animal community structure. *Trends in Ecology & Evolution,* **6,** 250-254 https://doi.org/10.1016/0169-5347(91)90071-5

Old, J. M., Hunter, N. & Wolfenden, J. unpublished. The preferred habitat, burrow characteristics and levels of mange in a population of bare-nosed wombats (*Vombatus ursinus*).

Palme, R. 2019. Non-invasive measurement of glucocorticoids: Advances and problems. *Physiology & behavior,* **199,** 229-243 https://doi.org/10.1016/j.physbeh.2018.11.021

Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S. & Möstl, E. 2005. Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. *Annals of the New York Academy of Sciences,* **1040,** 162-171

Raouf, S. A., Smith, L. C., Brown, M. B., Wingfield, J. C. & Brown, C. R. 2006. Glucocorticoid hormone levels increase with group size and parasite load in cliff swallows. *Animal Behaviour,* **71,** 39-48 https://doi.org/10.1016/j.anbehav.2005.03.027

Ruykys, L., Taggart, D. A., Breed, W. G. & Schultz, D. 2009. Sarcoptic mange in southern hairy-nosed wombats (*Lasiorhinus latifrons*): distribution and prevalence in the Murraylands of South Australia. *Australian journal of zoology,* **57,** 129-138

Sengupta, C., Old, J. M. & Narayan, E. Under Review. Testing the environmental decay of faecal cortisol metabolites in bare-nosed wombats (*Vombatus ursinus*) *General and Comparative Endocrinology,*

Sheriff, M. J., Dantzer, B., Delehanty, B., Palme, R. & Boonstra, R. 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia,* **166,** 869-887

Skerratt, L., Martin, R. & Handasyde, K. 1998. Sarcoptic mange in wombats. *Australian veterinary journal.* Victoria,Australia.

Skerratt, L. F. 1998. Diseases and parasites of the common wombat *Vombatus ursinus* in the Healesville area of Victoria. Surrey Beatty & Sons.

Skerratt, L. F. 2001. Sarcoptic mange in the common wombat, *Vombatus ursinus* (Shaw, 1800).

Skerratt, L. F., Skerratt, J. H. L., Banks, S., Martin, R. & Handasyde, K. 2004. Aspects of the ecology of common wombats (*Vombatus ursinus*) at high density on pastoral land in Victoria. *Australian Journal of Zoology,* **52,** 303-330 https://doi.org/10.1071/ZO02061

Sloane, M., Sunnucks, P., Alpers, D., Beheregaray, L. & Taylor, A. 2000. Highly reliable genetic identification of individual northern hairy-nosed wombats from single remotely collected hairs: a feasible censusing method. *Molecular Ecology,* **9,** 1233-1240 https://doi.org/10.1046/j.1365-294x.2000.00993.x

Vander Haegen, W. M., Orth, G. R., Johnston, A. N. & Linders, M. J. 2018. Endemic diseases affect population dynamics of tree squirrels in contrasting landscapes. *Journal of Wildlife Management,* **82,** 328-343 https://doi.org/10.1002/jwmg.21383

Walker, F. M., Sunnucks, P. & Taylor, A. C. 2006. Genotyping of "captured" hairs reveals burrow-use and ranging behavior of southern hairy-nosed wombats. *Journal of Mammalogy,* **87,** 690-699 https://doi.org/10.1644/05-MAMM-A-287R2.1

Wells, R. 1978. Field Observations of the Hairy-Nosed Wombat, *Lasiorhinus latifrons* (Owen). *Wildlife Research,* **5,** 299-303

Whitten, L., Brockman, D. & Stavisky, R. 1998. Recent Advances in Noninvasive Techniques to Monitor Hormone-Behavior Interactions. *American Journal of Physical Anthropology,* **Suppl 27,** 1-23 10.1002/(SICI)1096-8644(1998)107:27+3.0.CO;2-H

Zajac, A. M. & Conboy, G. A. 2012. Veterinary clinical parasitology, John Wiley & Sons.